EVALUATION OF SHORT-DAY ONION DOUBLED HAPLOID LINES

A Dissertation

by

RYAN LEE WALKER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Plant Breeding

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Chair of Committee,	Leonard M. Pike
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ABSTRACT

Evaluation of Short-day Onion Doubled Haploid Lines.

(December 2006)

Ryan Lee Walker, B.S.; M.S., Brigham Young University

Chair of Advisory Committee: Dr. Leonard M. Pike

Molecular marker analysis of seven putative onion (*Allium cepa*) doubled haploid (DH) lines developed at Texas A&M University was conducted to verify genetic homozygosity. Analysis was also conducted on five equivalent conventional inbred lines, breeding lines developed from the same parental crosses as the DH lines, and the original parent lines. The markers have revealed polymorphisms within the parental lines and the conventional inbreds, but not in the DH lines. We can conclude therefore that these seven lines are true DH lines. Performance of these DH lines was tested in two field locations and compared to commercial check lines. Bulbs from the various crosses were evaluated for eight bulb traits: diameter, height, centers/bulb, ring thickness, number of rings/bulb, bulb weight, soluble solids content, and pungency. Some crosses were detected that yielded significantly greater bulb weight than the check lines. However, these lines also had significantly greater numbers of centers per bulb. To test how these lines would perform in a breeding program, two full diallel analyses were conducted according to Griffing's Model I, Method 1. The first consisted of a four parent diallel cross using two red DH lines and two yellow DH lines. Bulbs from the

various crosses were evaluated for the same eight bulb traits mentioned above. Significant variation was detected for genotypic, general combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (MAT), and nonmaternal (NMAT) effects for all traits except number of rings/bulb, soluble solids content, and pungency. Significant environmental effects were only detected with number of centers per bulb. The second diallel analysis, a four parent diallel with two DH lines and two inbred lines from the breeding program, showed significant variation for the same effects for all traits except soluble solids content. Generally, GCA effects were more important than SCA effects in explaining the variation observed between crosses. For all traits GCA and SCA were always larger than the reciprocal effects (divided into maternal and nonmaternal components).

DEDICATION

I would like to dedicate this dissertation to my beautiful wife and our three children. Thank you for your love, support, and patience.

ACKNOWLEDGMENTS

I would like to recognize the support and help that I have received from each of the members of my committee. Their expertise has been very helpful in performing this research. Dr. Pike has been my mentor and teacher in the breeding program and I feel very fortunate to have been able to work with him.

I would also like to recognize the support that has been given to me by people not on my committee. Monica Menz served on my committee almost till the end, and has been a great support with the molecular work. Without her help and support the lab work would not have been possible. For help with the greenhouse work and seed cleaning, I would like to thank Travis Faske, Michael Faries, and the various students who assisted with that work. For assistance with the field trials, I would like to thank Daniel Leskovar and Rick Jones. I could not have done the work without the assistance of their research groups.

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CHAPTER I

INTRODUCTION

Onion (Allium *cepa* L.) is an important vegetable crop grown and used in many areas of the world. Despite its widespread cultivation and economic importance, limited genetic and molecular information is available to aid breeding efforts. The two characteristics of onions that make genetic analysis difficult are 1) the difficulty in developing populations for study and 2) the extremely large genome (Sparrow and Miksche, 1961). The development of genetic populations is hampered by the severe inbreeding depression of onion (Jones and Davis, 1944) and the biennial growth habit of the plant.

Despite these obstacles, molecular marker identification and mapping studies in onion have emerged in recent years. Although the emergence of these new marker techniques and maps provides a great resource for onion genetic studies, not all of the methods are of equal use in a breeding program. Nevertheless, the availability of these resources provides an opportunity for further testing in new populations and with new applications.

The introduction of doubled haploid (DH) lines provides a unique opportunity to create populations for genetic studies. Doubled haploid lines have been created both from spontaneous and induced doubling of haploid plants. One issue that must be

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This thesis follows the style of the Journal of the American Society for Horticulture Science.

investigated is the genetic stability and homozygosity of DH lines. Confirming the homozygosity of spontaneously doubled DH lines is especially important to ensure that these lines did not develop from maternal tissue (Geoffriau et al., 1997b). Published reports that have looked at this aspect in onion have used isozyme and random amplified polymorphic DNA (RAPD) markers to confirm genetic uniformity and to assess the stability of the lines (Bohanec et al., 1995; Campion et al., 1995; Javornik et al., 1998). Although RAPD markers are a quick and inexpensive marker type, their repeatability has been an issue that has limited their usefulness (Jones et al., 1997). The ability of more reliable marker types to confirm genetic uniformity of DH lines would be useful.

Two promising marker techniques are available in onion: sequence-tagged microsatellite (STMS) and amplified fragment polymorphism (AFLP) markers. Fischer and Bachmann (2000) determined that the STMS markers they developed were useful for genotyping and determining genetic relationships in onion. The AFLP markers developed by van Heusden et al. (2000b) were not very polymorphic between onion accessions; however, the high number of available markers still gives this technique promise. The ability of either of these marker types to detect remnant heterozygosity within a cultivar or breeding line, polymorphism between plants of the same line, has not been tested.

Another aspect of DH lines in onion that has received scant attention in the literature is the performance of DH lines under field conditions and their usefulness in a breeding program. Although Muren (1989) discusses a potential for rapid inbred development, published reports on DH lines have focused on their development rather

than on their use. More research is needed to determine the utility of DH lines in a breeding program.

Seven DH lines were developed at Texas A&M University from four different short-day F_2 and F_2M populations following the procedure described by Kim et al. (2004). Although root tip squashes of each line showed them to be diploid, and the stability and uniformity of the lines was confirmed phenotypically over several generations, no molecular analysis has been performed on these lines. The objectives of this research are to 1) test the suitability of the STMS and AFLP marker techniques for use with the DH lines developed at Texas A&M University, 2) to evaluate the value of DH lines for use as parents in a breeding program, and 3) to compare DH lines to conventional inbred lines as parents in a breeding program.

CHAPTER II

LITERATURE REVIEW

Onion (Allium *cepa* L.) is a bulbing vegetable crop grown in many areas of the world. It has been used as a food source for thousands of years, and is thought to have originated in the area around Afghanistan (Vavilov, 1951). Although the exact origin remains somewhat of a mystery, recent research has identified A. *vavilovi* as its closest wild relative (Bradeen and Havey, 1995). Onion is also very important in the U.S., ranking third in overall value among vegetables produced (U.S. Department of Agriculture, 2005). However, despite its economic importance, the amount of genetic and molecular information available to aid breeding efforts in onion remains quite limited. This is especially true if compared to tomato and other leading vegetable crops. Genetic analysis in onion has been hampered by 1) severe inbreeding depression (Jones and Davis, 1944), 2) the extremely large genome (Sparrow and Miksche, 1961), and 3) the biennial life cycle of onion.

Despite these obstacles, molecular marker identification and mapping studies in onion have emerged in recent years. Wilkie et al. (1993) reported the first molecular marker work in onion, using random amplified polymorphic DNA (RAPD) markers to assess variability among several *Allium* species. King et al. (1998) generated a loose map of onion using restriction fragment length polymorphism (RFLP) markers. They also determined that the large genome in onion is likely due to intrachromosomal duplication, confirming the conclusion reached in a previous cytological paper

(Karavanov and Iordanskii, 1973). Another map, based on amplified fragment length polymorphism (AFLP) markers, was published shortly afterward (van Heusden et al., 2000a). Sequence-tagged microsatellite (STMS) markers were developed that may be useful to determine intraspecific relatedness (Fischer and Bachmann, 2000) and expressed sequence tags (ESTs) were discovered that can also be used for this purpose (McCallum et al., 2001). More recently, a map consisting of single nucleotide polymorphism (SNP), indel, and simple sequence repeat (SSR) markers has been published (Jakše et al., 2005).

These advances in onion molecular genetics may prove useful to breeders for 1) the mapping of traits of interest, 2) to classify germplasm and identify new sources of genetic variation, and 3) to evaluate the relationship of expected parental contribution to observed parental contribution in onion. Molecular markers have been shown to be effective for estimating coefficients of coancestry (f_{ij}) by determining parental contribution to offspring (Bernardo et al., 2000). The comparison of expected parental contribution to observed parental contribution in maize has shown that F_2s may inherit up to 79% of their marker alleles from one parent, and it may be even higher for DH lines developed from F_2 plants (Bernardo and Kahler, 2001).

Hybrid breeding in onion became feasible on a commercial level after the identification of a male sterile onion line (Jones and Emsweller, 1937), and initial high parent heterosis estimates for yield were -26% to 192% (Jones and Davis, 1944). However, with the subsequent improvement of inbreds, heterosis estimates have been depressed. More recent estimates give HPH estimates for yield of 12% to 20% for long

day inbreds and ≤ 50 % with short day inbreds (Hosfield et al., 1977b; Netrapal and Singh, 1999). Although these results are still encouraging, it must be remembered that part of the heterosis observed when inbred parents are crossed is due to the recovery of lost vigor due to inbreeding depression. Yield performance estimates comparing hybrids to OPs range from -15% to 9% (Dowker and Gordon, 1983) to 33% to 52% (Aghora and Pathak, 1991). However, in the U.S. uniformity is more important than yield (Pike, 1986). For this reason, among others, U.S. breeding programs continue to emphasize hybrid production.

In hybrid development, onion breeders strive to maximize uniformity while maintaining a certain level of vigor in the inbred parents. Because of severe inbreeding depression, parents used to create hybrids are not true inbreds: usually a maximum of three selfing cycles are possible, followed by recurrent selection (Pike, 1986). After desirable parents are identified, male sterile pairs are created for inbred parents by backcrossing. Although this system works well for breeding and hybrid production, it is not conducive to genetic studies. The heterosis estimates obtained by using these lines would be underestimated due to remnant heterogeneity and heterozygosity in the male sterile parent and remnant heterozygosity in the male fertile parent. The only way to obtain unbiased estimates is to use homozygous parent lines.

The first report of the induction of haploid plants in onion used ovary culture (Muren, 1989). Later, ovule culture was attempted (Campion and Alloni, 1990; Keller, 1990) as well as immature flower culture (Keller, 1990). All three methods were compared by Campion et al. (1992) and the recommendation was to use both ovary and

immature flower culture. The gynogenic response in onion is relatively low, and numerous studies have been performed to increase this response. These reports can be categorized based on the factors evaluated in the study. Factors analyzed in attempts to improve the process include 1) media components (Bohanec et al., 1995; Campion et al., 1992; Jakše et al., 1996), 2) temperature (Hassandokht and Campion, 2002; Muren, 1989), and 3) flower age (Muren, 1989; Musial et al., 2001). However, it appears that the factor with the largest effect is the genotype of the mother plant. Therefore, the best approach to increasing gynogenic response in onion is to work with genotypes with a high response (Bohanec and Jakše, 1999). As a result, several studies looking at the variability in onion genotypes for gynogenic response were conducted (Bohanec and Jakše, 1999; Geoffriau et al., 1997b; Michalik et al., 2000). The potential for improving this response through selection was also evaluated using second cycle gynogenesis (Javornik et al., 1998). This study did achieve the highest response reported in the literature to date with just over 1 haploid embryo per cultured flower. Unfortunately, the other two highly responsive lines tested did not show much improvement in the second cycle.

Although induced chromosome doubling in haploids has been studied (Geoffriau et al., 1997a), most DH plants have relied on spontaneous chromosome doubling. One question that must be answered is the genetic stability and homozygosity of DH lines. Confirming the homozygosity of spontaneously doubled DH lines is especially important to ensure that these lines didn't develop from maternal tissue (Geoffriau et al., 1997b). Published reports that have looked at this aspect in onion have used isozyme

and random amplified polymorphic DNA (RAPD) markers to confirm genetic uniformity and to assess the stability of the lines (Bohanec et al., 1995; Campion et al., 1995; Javornik et al., 1998).

CHAPTER III

MOLECULAR EVALUATION

INTRODUCTION

Although onion (Allium cepa L.) is an important vegetable crop in many areas of the world, the amount of genetic and molecular information available is quite limited as compared to other important crop species. This lack of study is due in part to the long generation time and the subsequent time required to develop the desired populations for study. Other contributing factors include an extremely large genome (Sparrow and Miksche, 1961) and severe inbreeding depression (Jones and Davis, 1944).

The introduction of doubled haploid (DH) lines provides a unique opportunity to create populations for genetic studies. Doubled haploid lines have been created both from spontaneous and induced doubling of haploid plants. The induction of haploid plants in onion using ovary culture (Campion et al., 1992; Muren, 1989), ovule culture (Campion & Alloni, 1990; Campion et al., 1992; Keller, 1990), and immature flower culture (Campion et al., 1992; Keller, 1990) have been reported. The gynogenic response in onion is relatively low, and numerous studies have been performed to increase this response (Bohanec et al., 1995; Hassandokht and Campion, 2002; Jakše et al., 1996; Javornik et al., 1998; Musial et al., 2001). However, the factor with the largest effect seems to be the genotype of the mother plant. Therefore, the best approach to increasing gynogenic response in onion is to work with genotypes with a high response (Bohanec and Jakše, 1999). In fact the highest response reported in the literature,

118.3% (an average of more than one haploid per cultured flower), was obtained from a second cycle of gynogenesis from a highly responsive line (Javornik et al., 1998). Unfortunately, the other two highly responsive lines tested did not show much improvement in the second cycle.

One question that must be answered is the genetic stability and homozygosity of DH lines. Confirming the homozygosity of spontaneously doubled DH lines is especially important to ensure that these lines didn't develop from maternal tissue (Geoffriau et al., 1997b). Published reports that have addressed this concern in onion have used isozyme and random amplified polymorphic DNA (RAPD) markers to confirm genetic uniformity and to assess the stability of the lines (Bohanec et al., 1995; Campion et al., 1995; Javornik et al., 1998). Sequence-tagged microsatellite (STMS) markers have been developed that are useful for genotyping and determining genetic relationships in onion (Fischer and Bachmann, 2000). Amplified fragment polymorphism (AFLP) markers have also been developed in onion (van Heusden et al., 2000b). However, the ability of either of these marker types to detect remnant heterozygosity within a cultivar or breeding line, polymorphism between plants of the same line, has not been tested.

Seven DH lines were developed at Texas A&M University from five different short-day F_2 and F_2M populations following the procedure described by Kim et al. (2004). The original parental material of the DH lines are '1015' (US open-pollinated variety), '1025' (US open-pollinated variety), 'Ori' (Israeli open-pollinated variety), 'Ringer' (US open-pollinated variety), and 'Cardinal' (US hybrid). Although root tip

squashes of each line showed them to be diploid, and the stability and uniformity of the lines was confirmed phenotypically over several generations, no molecular analysis has been performed on these lines. The purpose of this research is test the utility of the STMS and AFLP marker techniques in differentiating between and confirming the uniformity of the DH lines developed at Texas A&M University.

MATERIALS AND METHODS

DNA Extraction

The protocol for DNA extraction was a modification of the method described by Williams and Ronald (1994). Approximately 1 g of leaf tissue was placed in a 1.5 ml microcentrifuge tube with 180 µl PEX buffer and a metal rod. Tissue was homogenized on a Genogrinder (2000; SPEX Certiprep Inc., Metuchen, NJ, USA) for 30 seconds. Samples were incubated at 65 ºC for 30 minutes in a water bath followed by centrifugation for 5 minutes at 13000 RPM. The supernatant (100 µl) was transferred to another microcentrifuge tube containing 100 µl isopropanol at room temperature. Tubes were inverted several times and then centrifuged again as described above. Supernatant was removed, 200 µl of cold 70% EtOH (v/v) was added, and samples were placed in a -20 ºC freezer for 1 hour. This wash was repeated, followed by removal of the ethanol, and the pellet was allowed to air dry for at least 15 minutes to allow evaporation of any residual ethanol. The final step in the process was the addition of 30 µl of TE buffer, 1 µl RNAse A, and incubation at 65 ºC for 15 minutes. Extracted DNA was quantified using a Fluorometer (TD-360; Turner Designs Inc., Sunnyvale, CA, USA) and then

diluted to a final concentration of 7.5 ng/µl for STMS analysis and 100 ng/µl for AFLP analysis using 0.5X TE buffer.

Marker Analysis

STMS analysis was performed according to Fischer and Bachmann (2000) with the following modifications: PCR reactions were scaled down to 15 µl and only 0.5 U Taq (Promega Corporation, USA) and 0.03 µM infrared-labeled primer were used in the reactions. PCR reactions were run on a GeneAmp PCR System (Applied Biosystems 2700; Foster City, CA, USA). PCR product (1 µl) was run on a 6 % acrylamide gel and read by a DNA Analysis System (Li-Cor 4200; Lincoln, NE, USA).

AFLP analysis was conducted following the protocol given by Vos et al. (1995) as modified by Klein et al. (2000). Dilution of pre-amplification, and the use of seven selective nucleotides (+3, +4) for the selective amplification, followed van Heusden et al. (2000b) because of the crop specificity. PCR product from the selective amplification was analyzed as described above.

RESULTS

STMS Analysis

To test the repeatability of this marker system, two DNA isolations per plant were taken from two plants in each DH line. These samples were screened with five of the STMS markers (AMS06, AMS08, AMS23, AMS25, and AMS26). One of the markers, AMS08, gave random bands that were not consistent between DNA isolations on the same plant or on different plants in the same line (data not shown). Repeated

tests confirmed that the marker would not work on this material. The other four markers gave consistent results between isolations from the same plant, and between plants of the same line. The markers were highly polymorphic between the different DH lines, differentiating between all lines except DH 6 and DH 8. In fact, AMS26 alone could distinguish all lines except DH 6 and DH 8 (Fig. 1).

Because the STMS markers were highly polymorphic in the repeatability tests, it was determined that they might be useful in confirming the uniformity of the DH lines. In order to accomplish this objective, the markers must be able to detect polymorphism within the original parent lines and the conventional inbreds, but not in the DH lines. Towards this end, tests were run on five separate individuals from each DH line and six individuals from both the conventional inbreds and the original parent lines. The four markers also detected polymorphisms within the conventional inbreds and the original parent lines, but not within any of the DH lines (Table 1).

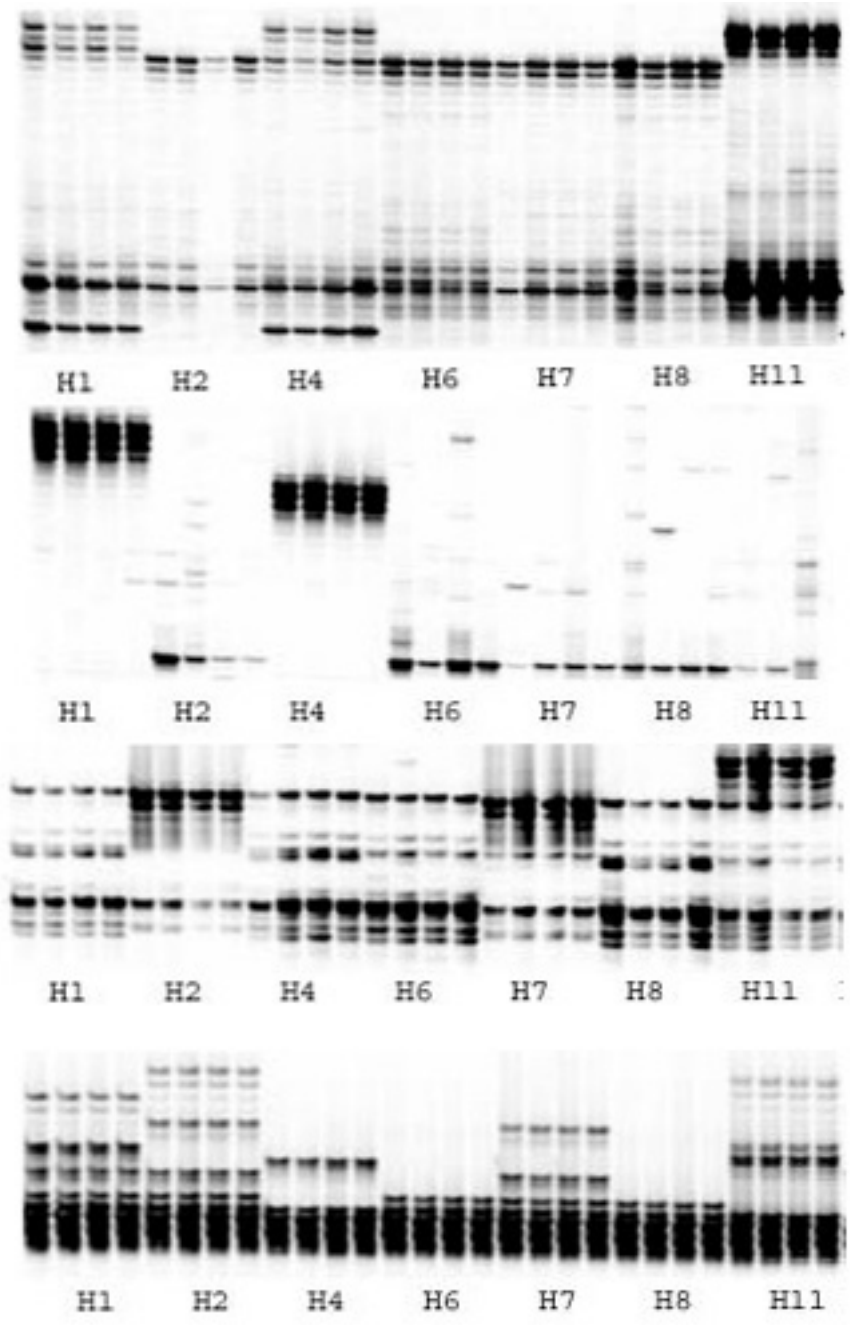

Fig. 1**.** Results of STMS markers AMS06, AMS23, AMS25, and AMS26 (from top to bottom) when run on the seven DH lines. Four lanes are shown for each DH line. Lanes 1 and 2 correspond to two samples taken from one plant and Lanes 3 and 4 correspond to two samples taken from an additional plant from the same line.

Table 1. Number of genotypes revealed by four STMS markers.

	Number of Genotypes[y]			
Line[z]	AMS06	AMS23	AMS25	AMS26
DH 1	1	1	1	1
DH 2	1	1	1	1
DH 4	1	1	1	1
DH 6	1	1	1	1
DH 7	1	1	1	1
DH 8	1	1	1	1
DH 11	1	1	1	1
I 4	2	2	1	3
I 11 A	2	2	2	2
I 11 B	2	1	2	4
I 1,6,8	2	2	2	2
I 2,7	2	3	1	3
P 2,7	3	1	1	3
P 1,2,4,6,7,8	3	2	1	2
P 4	2	2	2	2
P 1,6,8,11	3	3	3	5
P 11	1	1	3	3

[z]DH = doubled haploid, I = conventional inbred, and P = parent line.
[y]Out of five (DH) or six (I, P) plants.

AFLP Analysis

To verify the presence or absence of bands within the lines, two isolations per plant and two plants per line were tested for each DH line. In addition, two bulks of ten plants each were also run for the conventional inbreds and the parent lines to assess the ability of the technique to detect differences between the DH lines and these other groups. Twenty four AFLP marker combinations were run on the samples (see appendix). Although the rate of polymorphism was very low, a few bands were found which differentiated between the DH lines. A combination of four markers could distinguish between all seven DH lines (Table 2). However, not as much polymorphism

was detected between the DH lines and the other bulks as was expected. For this reason, no further testing was performed with this type of marker.

Table 2. AFLP primer group which can differentiate between DH lines.

Selective Primers		Size (bp)	DH Lines						
			H1	H2	H4	H6	H7	H8	H11
E-CAA	M-CACG	229	–	+	+	+	+	+	+
E-CAA	M-CACG	447	+	–	–	+	–	–	+
E-CAA	M-CACG	449	–	+	+	–	+	+	–
E-TGA	M-CACG	359	–	+	–	+	–	+	–
E-CTG	M-CCCT	278	+	–	–	+	–	+	+
E-ACT	M-CCCT	150, 151	+	+	–	+	+	+	+

DISCUSSION

STMS Analysis

The marker profile and repeatability of the STMS markers described by Fischer and Bachmann (2000) was unknown for this material. It was also unknown if they would be polymorphic enough to differentiate between the lines. Four of the five markers proved to be repeatable and very polymorphic. The reason that one marker, AMS08, failed to amplify is unknown. Perhaps it was because the microsatellite locus this primer set amplifies was not present in this material.

Although in their publication Fischer and Bachmann (2000) described these markers as highly polymorphic, they were testing them on accessions and landraces. It was somewhat surprising that they maintained such a high level of polymorphism in the material tested. This result is likely due to the diversity that existed in the parents used in the initial crosses to create the populations from which the DH lines were created.

The STMS markers were very useful in detecting remnant heterogeneity within both the original parents and the conventional inbred lines. It would be interesting to test these markers to determine if they would be able to verify the gynogenic origin of DH lines. To accomplish this objective, they would need to yield unique profiles for the mother plant and the DH line. Due to the fact that these DH lines were created years ago, no DNA was available from the mother plants for testing this hypothesis with this material.

AFLP Analysis

Although twenty four marker combinations is a small amount of the possible combinations that could be run with AFLP, this does give some indication of the performance of AFLPs in this material. These findings are in agreement with reported polymorphism detected by AFLP in cultivated onion (van Heusden et al., 2000b). It was hoped that this technique would provide they enough information to compare a DH line to its corresponding conventional inbred and their original parent lines. However, the low frequency of polymorphisms observed did not allow such comparisons to be made.

Comparison of Methods

The STMS markers were very polymorphic, demonstrating their utility for confirming pedigrees and detecting contamination in this material. AFLP markers, by contrast, were not very informative in this material. This result is likely due to the nature of the marker type. The STMS markers are targeted on a highly variable microsatellite region of the genome. AFLPs on the other hand are not a targeted technique, providing instead a scan of the genome. Another factor to consider is the

nature of the genome under study. The large genome in onion is due to intrachromosomal duplication (Karavanov and Iordanskii, 1973; King et al., 1998) and consists largely of repetitive fractions (Stack and Comings, 1979). Because of these factors, the targeted microsatellite approach yields a quicker, more informative result when attempting to detect remnant heterozygosity within these lines.

CHAPTER IV

DOUBLED HAPLOID DIALLEL

INTRODUCTION

Through careful selection and controlled mating, plant breeders utilize genetic variation to improve a trait of interest. To be successful, there must be variation within the breeding population and the breeder must be able to select those individuals within the population that have superior alleles for the trait. The methods used vary depending on the mode of gene action and the heritability of the trait.

The diallel mating design devised by Griffing (1956) is a valuable tool for breeders. It allows the partitioning of the variance among crosses into genetic and environmental components. The variation due to genetic components can be further subdivided into main effects (general and specific combining abilities) and interactions (reciprocal effects). The general and specific combining abilities are related to additive and dominant gene action, respectively. The reciprocal effects can be further subdivided into maternal and nonmaternal effects, as described by Cockerham (1963). Baker (1978) concluded that diallels should only be used to estimate combining ability effects, citing the difficulty of meeting the assumptions of independent distribution of genes in the parents and no epistasis. Christie and Shattuck (1992) stated that the estimation of combining ability effects requires no assumptions about a reference population. This makes it valuable for crops in which it is difficult to create genetic populations for study.

The knowledge of combining ability effects allows breeders to determine the best breeding and selection strategies to use in trait improvement.

General combining ability predominates for all traits reported in onion (Hosfield et al., 1976; Hosfield et al., 1977a; Krueger et al., 1989). Reciprocal effects, although not very common in plants (Hosfield et al., 1976), have been significant for several traits in each of the above-mentioned reports on onion. Hosfield et al. (1977a) found that fewer traits showed significant reciprocal effects after extending their study over two years. They attributed this to overestimation of the effects in their first experiment, although they could not eliminate bias due to different numbers of parents in the two experiments. However, even in their second experiment three out of eight traits retained significant reciprocal effects. It is not known exactly why reciprocal effects continue to be important in onion; however, this demonstrates the importance of choosing experimental designs capable of measuring these effects.

Onion doubled haploid (DH) lines were first reported in the literature by Muren (1989). The creation of completely homozygous lines is something of a paradox due to the severe inbreeding depression in onion. It is tempting to speculate that the technique provides strong selection against deleterious recessive alleles due to the low regeneration frequency (1.3 % average) of onion (Geoffriau et al., 1997b) and because there is no detectable loss of vigor in the selfed progeny of DH lines. Although this may be partly true, the complete story is more complex. A second cycle of gynogenesis only showed considerable increase in regeneration frequency in one out of the three lines included in a study by Javornik et al. (1998). Nevertheless, the existence of DH lines provides a

unique opportunity to look at the potential of these lines for use in a breeding program. However, no information is available in the literature about the performance of onion DH lines or their hybrids.

The objectives of this experiment are 1) to compare the mean performance of onion DH lines and their hybrids to commercial checks, and 2) to estimate the combining ability effects of DH lines to evaluate their potential in a breeding program.

MATERIALS AND METHODS

Plant Material

Seven DH lines were developed at Texas A&M University from five different short-day F_2 and F_2M populations following the procedure described by Kim et al. (2004). The original parental material of the DH lines are '1015' (US open-pollinated variety), '1025' (US open-pollinated variety), 'Ori' (Israeli open-pollinated variety), 'Ringer' (US open-pollinated variety), and 'Cardinal' (US hybrid). Pedigrees are as follows: 'Ori' X 'Ringer' (H1), 'Ringer' X 'Ori' (H6, H8), '1015' X 'Ori' (H2, H7), 'Ori' X 'Cardinal' (H4), and '1025' X 'Ringer' (H11). Although two pairs of DH lines (H2, H7; and H6, H8) came from the same mother plant, they are phenotypically distinct from one another in both cases.

Crosses

A complete diallel crossing scheme involving seven DH lines was attempted as described by Griffing (1956). However, not all of the crosses could be made because of unusually warm winter weather which reduced vernalization and the resultant flowering

of the parent plants. Attempts were made in two successive years to vernalize non-bolting onion plants. The first year, non-bolting plants were placed in a cold room (40 ºC) for two to four weeks. Although some bolting did occur, the scapes died shortly after emergence from the bulb. The second year, non-bolting onions were harvested and the bulbs were cut in half and grown in a growth chamber. Unfortunately, the onions had to be removed from the growth chamber shortly after new leaves emerged from the bulbs and the young plants died shortly after being transferred to the greenhouse. The failure to induce bolting in three lines (1, 4, and 7) resulted in crosses being made with only a few plants per line. As a result, a complete diallel was only obtained for the other four lines (2, 6, 8, and 11).

Experimental Design

Seeds from all crosses (Table 3) were sown in 288-well transplant trays (Dillen Products Inc., USA) on November 6th and grown in the greenhouse until transplanting. Transplants were arranged in an α-lattice incomplete block design (Patterson and Williams, 1976) with three reps per entry. Plots were ten feet long with two foot alleys. Two rows, twelve inches apart, were planted in each plot with plants spaced five inches apart.

The trial in Uvalde, TX was transplanted on January 14th and 15th. Plots were placed in rows eighty inches apart on low pressure drip tape (Netafim USA, USA). The trial in La Mesa, NM was planted on February 16th and 17th under furrow irrigation.

Because rows in the field were forty inches apart, experimental plots were planted on every other row. A single row of Texas Early White was planted in the rows between plots.

Table 3. List of crosses between all doubled haploid lines.

♀ | ♂	H1	H2	H4	H6	H7	H8	H11
H1	1	5	10				24
H2	2	6		15		20	25
H4			11	16			
H6		7	12	17		21	26
H7	29				3	30	
H8		8	13	18	31	22	27
H11	4	9	14	19		23	28

Phenotypic Evaluation

Plots were harvested at each location at maturity (>80% tops down). Up to ten bulbs (see appendix) were selected from each plot and were measured for eight traits: bulb diameter (BD), bulb height (BH), number of centers per bulb (C/B), number of rings per bulb (R/B), ring thickness (RT), bulb weight (BW), soluble solids content (SSC), and pyruvic acid content (PA). Measurement for BD was taken at the bulbs widest point; if the bulb was an obvious double, an average of two measurements taken on opposite sides was used. The measurement for BH was taken immediately adjacent to the root mass at the base and the dried leaves at the top of the bulb. For C/B, bulbs with all growing points located within a center diameter of 3.0 cm were considered single center. If the bulb did not meet this standard, the total number of growing points was tallied. RB was a measurement of rings that continued at least half way around the

bulb. RT was measured on the second ring of the bulb. If the ring varied in thickness, the measurement was taken at a location that was considered representative of the average ring thickness for the bulb. Bulbs were weighed individually to obtain BW. SSC and PA were measured from frozen juice as described below.

Bulbs were cut horizontally into two halves, with a 5 mm slice removed from the top half. The slice was placed in a plastic baggie and crushed with a hand press. The sample was then left at room temperature for 20-30 minutes and then placed in a freezer at -20 ºC. Juice from all samples was analyzed for soluble solids content using a hand refractometer (10430; American Optical Corp., Buffalo, NY, USA) and pyruvic acid content was measured according to Schwimmer and Weston (1961) as modified by Yoo and Pike (1999).

Statistical Analysis

Individual plant data were used to calculate mean performance of the seven DH lines, the 12 hybrid crosses and their reciprocals, and four commercial checks; 'Legend', two experimental hybrids made with 'Legend', and 'Early Sunrise'. The data were analyzed in SAS 9.1.3® using proc glm and proc mixed. Efficiency (e) of the design was determined by the formula:

$$e = \left(\frac{SED_{RBD}}{SED_{IB}} \right)^2$$

where

SED_{RBD} is the standard error of the difference of means using the RBD design;

SED_{IB} is the standard error of the difference of means using the IB design

The adjusted means from the design that gave the lowest SED values were used to determine mean performance and to compare to the commercial checks.

The combining ability analyses were conducted with plot averages from all possible crosses between four DH lines (2, 6, 8, and 11) designated as parents 1 through 4 respectively. The data were analyzed as an all fixed model (Model 1 Method 1) as described by Griffing (1956) using the program Diallel-SAS05 (Zhang et al., 2005). The program analyzes the data using unadjusted means with a RCB design. The data were analyzed in each individual environment and in a combined environment analysis. Homogeneity of variance tests between the two environments were conducted as described by Snedecor and Cochran (1967). To determine the relative importance of GCA and SCA in predicting hybrid performance for these traits, the following formula was used (Baker, 1978):

$$\frac{2g_i}{2g_i + s_{ij}}$$

where

g_i is the mean square of the GCA effect in the combining ability ANOVA;

s_{ij} is the mean square of the SCA effect in the combining ability ANOVA

RESULTS

Locations

Trait means between the two locations were comparable for the most part (Table 4). Trait means were generally higher in Uvalde than in La Mesa, except for RB and SSC. The variance was generally greater in La Mesa.

Table 4. Means and $LSD_{0.05}$ for bulb traits measured at two locations.

	Uvalde, TX		La Mesa, NM	
Character	**General Mean**	**LSD**	**General Mean**	**LSD**
Diameter (in.)	3.43	0.32	3.21	0.36
Height (in.)	2.21	0.16	2.10	0.17
Centers/bulb	1.91	0.48	1.54	0.41
Rings/bulb	7.02	0.66	7.36	0.69
Ring thickness (in.)	0.203	0.027	0.196	0.029
Bulb weight (g)	256.62	57.43	216.12	57.04
Soluble Solids (brix)	4.47	1.04	5.70	1.32
Pungency (µM pyruvate/ml)	5.09	1.05	4.32	1.03

Mean Performance

Efficiency values were very consistent between traits and were similar between locations. The incomplete block (IB) design used by proc mixed was more efficient at partitioning the variance, evidenced by the lower SED values obtained and the efficiency values greater than unity, than the randomized complete block (RCB) design used by proc glm for all traits tested (Table 5).

The mean performance of the DH lines was statistically similar to the commercial checks, with a few exceptions (Tables 6, 7). In Uvalde, four lines had lower BH (1, 6, 17, and 22), three lines had higher C/B (17, 22, and 28), one line had less R/B (6), two lines had lower RT (17 and 28), one line had higher RT (11), one line had lower BW (22), and three lines had higher PA (1, 17, and 22). In La Mesa, one line had lower BD (11), four lines had lower BH (1, 11, 17, and 22), two lines had lower BW (1 and 11), one line had higher SSC (1), and one line had higher PA (1).

Many of the hybrids were statistically different from the commercial checks for the traits measured (Tables 5, 6). In Uvalde, ten hybrids had higher BD (4, 5, 10, 13, 23, 24, 26, 27, 29, and 31), eleven had higher C/B (4, 5, 7, 9, 14, 18, 19, 21, 23, 24, and 26)

Table 5. Comparison of SED values given by analysis of experiment as a randomized complete block (RCB) and an α-lattice (IB).

	Uvalde, TX			La Mesa, NM		
Character	**RCB**	**IB**	**Efficiency[z]**	**RCB**	**IB**	**Efficiency**
Diameter (in.)	0.49	0.16	29.35	0.49	0.18	19.09
Height (in.)	0.25	0.08	29.64	0.24	0.09	18.93
Centers/bulb	0.73	0.24	29.03	0.58	0.21	20.33
Rings/bulb	1.02	0.33	29.30	0.92	0.35	18.28
Ring thickness (in.)	0.04	0.01	29.29	0.04	0.01	18.63
Bulb weight (g)	88.79	28.71	29.57	76.23	29.10	17.97
Soluble Solids (brix)	1.64	0.52	31.10	1.76	0.68	17.74
Pungency (μM pyruvate/ml)	1.60	0.52	28.39	1.39	0.53	18.21

[z]Measured as $e=(SED_{RCB}/SED_{IB})^2$ where the numerator and denominator are the standard error of the difference of means using the RCB and IB designs respectively.

one had lower R/B (10), one had higher R/B (4), four had lower RT (9, 14, 19, and 26), four had higher RT (10, 13, 25, and 31), six had higher BW (4, 10, 13, 23, 24, and 29), one had lower SSC (10), and six had higher PA (4, 5, 7, 15, 18, and 21). In La Mesa, two hybrids had higher BD (23, 24), nine had lower BH (2, 4, 7, 8, 10, 18, 20, 21, 30, and 31), nine had higher C/B (4, 9, 14, 16, 19, 23, 24, 26, and 27), two had lower RT (19 and 26), three had higher RT (8, 10, and 13), one had lower BW (21), one had higher BW (23), one had higher SSC (25), and one had higher PA (16).

Table 6. Mean performance of entries grown in Uvalde, TX.

Entry	Parent 1	Parent 2	Bulb Trait[z]							
			BD	BH	C/B	R/B	RT	BW	SSC	PA
1	1	1	3.01	1.88	1.66	7.62	0.191	168.35	4.62	8.53
2	2	1	3.38	2.54	1.23	6.17	0.228	241.10	5.56	5.34
3	2	2	3.27	2.22	1.29	6.74	0.167	231.08	5.01	3.66
4	11	1	4.38	2.61	2.94	8.35	0.211	474.49	3.44	6.54
5	1	2	3.76	2.38	2.04	7.24	0.231	309.62	4.52	6.53
6	4	4	2.89	2.19	1.36	5.46	0.182	176.23	4.23	5.28
7	6	2	3.54	2.22	2.91	8.10	0.183	258.90	4.24	6.05
8	8	2	3.46	2.03	1.65	7.47	0.230	229.56	4.57	5.09
9	11	2	3.42	2.30	2.48	6.87	0.149	262.64	4.57	2.82
10	1	4	3.73	2.20	1.61	5.69	0.290	323.96	2.10	5.74
11	6	6	3.26	2.30	1.06	6.21	0.252	229.14	5.64	4.32
12	6	4	3.47	2.16	1.48	6.40	0.213	255.44	4.56	4.51
13	7	4	3.86	2.27	1.75	6.74	0.288	324.03	5.65	3.66
14	8	4	3.36	2.32	2.66	6.63	0.129	236.44	5.97	3.23
15	2	6	3.44	2.24	1.58	6.09	0.219	238.77	5.64	6.93
16	4	6	3.23	2.27	1.38	6.66	0.215	214.91	5.39	5.47
17	7	7	2.99	1.88	2.19	7.88	0.157	166.13	3.31	9.46
18	8	6	2.99	1.72	2.07	6.85	0.208	156.37	2.71	8.44
19	11	6	2.93	1.91	2.46	7.22	0.156	197.88	4.20	4.88
20	2	8	3.35	1.95	1.39	7.18	0.217	213.97	4.11	4.30
21	6	8	2.96	1.76	2.16	7.10	0.166	157.45	4.08	10.09
22	8	8	3.05	1.77	2.42	7.40	0.183	154.53	4.08	6.11
23	11	8	4.18	2.27	2.78	7.58	0.199	415.68	3.75	3.47
24	1	11	4.06	2.39	3.90	7.57	0.173	375.00	5.33	3.87
25	2	11	3.40	2.32	1.33	6.30	0.250	245.46	3.48	4.64
26	6	11	3.69	2.26	2.91	7.23	0.159	306.04	4.58	4.25
27	8	11	3.73	2.20	1.82	7.97	0.203	286.41	4.87	2.79
28	11	11	3.25	2.29	2.41	6.37	0.139	230.05	4.01	3.70
29	7	1	4.07	2.52	1.58	7.20	0.219	397.71	4.73	4.73
30	7	8	3.41	2.19	1.40	7.19	0.240	240.12	3.99	4.29
31	8	7	3.70	2.07	1.73	7.26	0.244	295.26	4.25	5.28
32	'Legend'		3.36	2.58	1.16	7.03	0.214	264.02	4.53	3.35
33	Exp. Hybrid 1		3.26	2.37	1.45	7.65	0.187	244.53	6.32	3.15
34	Exp. Hybrid 2		3.28	2.52	1.21	7.49	0.198	246.99	3.36	2.70
35	'Early Sunrise'		3.02	2.44	1.30	6.68	0.204	213.37	4.99	4.94
LSD			0.31	0.16	0.47	0.65	0.027	56.28	1.02	1.03
Mean			3.43	2.21	1.91	7.02	0.203	256.62	4.47	5.09

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

Table 7. Mean performance of entries grown in La Mesa, NM.

Entry	Parent 1	Parent 2	Bulb Trait[z]							
			BD	BH	C/B	R/B	RT	BW	SSC	PA
1	1	1	2.50	1.67	1.18	7.74	0.165	98.96	7.76	6.86
2	2	1	2.96	1.97	1.74	6.55	0.227	161.28	6.57	6.39
3	2	2	3.27	2.35	1.06	7.27	0.174	228.06	4.16	3.91
4	11	1	3.09	2.04	1.91	7.33	0.161	240.62	5.52	2.99
5	1	2	3.41	2.19	1.25	8.17	0.207	235.49	7.36	4.69
6	4	4	2.95	2.12	1.06	7.37	0.206	164.34	5.39	4.37
7	6	2	3.15	2.05	1.48	7.46	0.186	191.88	5.11	4.33
8	8	2	3.30	1.93	1.23	7.34	0.233	201.15	5.64	3.56
9	11	2	3.21	2.24	1.94	7.35	0.156	235.99	3.93	2.64
10	1	4	3.06	1.77	1.11	6.92	0.237	160.69	5.54	6.45
11	6	6	2.15	1.58	0.99	6.02	0.187	82.07	4.46	5.49
12	6	4	3.24	2.12	1.31	7.17	0.205	216.76	5.25	4.04
13	7	4	3.55	2.13	0.99	6.37	0.295	270.35	7.07	6.27
14	8	4	2.87	2.17	1.97	6.58	0.148	181.31	6.30	2.70
15	2	6	3.23	2.13	1.57	8.02	0.185	199.21	4.87	4.37
16	4	6	3.51	2.25	1.81	7.20	0.221	257.74	5.43	7.29
17	7	7	2.88	1.95	1.25	7.30	0.179	153.92	6.03	3.85
18	8	6	3.26	1.82	1.41	7.15	0.225	191.39	5.07	4.10
19	11	6	3.65	2.26	2.83	8.51	0.142	300.80	5.20	2.58
20	2	8	3.22	1.96	1.13	7.70	0.237	189.08	4.03	4.34
21	6	8	2.73	1.75	1.70	6.93	0.184	124.24	4.92	4.32
22	8	8	3.23	1.82	1.47	7.08	0.226	190.42	6.47	4.13
23	11	8	4.27	2.33	2.76	8.52	0.185	416.23	6.28	2.92
24	1	11	3.88	2.27	2.48	8.29	0.171	322.52	6.22	4.22
25	2	11	2.91	2.10	1.07	7.28	0.201	182.03	7.97	5.37
26	6	11	3.50	2.23	2.56	7.85	0.142	266.40	4.63	4.01
27	8	11	3.65	2.22	1.97	7.46	0.203	285.38	6.04	3.22
28	11	11	2.76	2.14	1.68	6.74	0.160	163.09	5.82	3.84
29	7	1	3.64	2.32	1.00	7.88	0.229	280.75	5.67	4.49
30	7	8	3.38	2.05	1.68	7.62	0.190	217.87	6.76	4.01
31	8	7	3.34	1.96	1.28	7.15	0.227	213.10	6.40	3.39
32	'Legend'		3.07	2.48	1.09	7.23	0.200	230.57	4.54	3.45
33	Exp. Hybrid 1		3.17	2.43	1.30	7.64	0.176	241.87	6.33	5.74
34	Exp. Hybrid 2		3.44	2.53	1.13	7.85	0.190	286.26	4.59	2.90
35	'Early Sunrise'		2.86	2.24	1.36	6.41	0.200	182.24	5.99	4.09
LSD			0.36	0.17	0.41	0.69	0.029	57.04	1.32	1.03
Mean			3.22	2.06	1.58	7.37	0.197	213.65	5.74	4.36

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

Combining Ability

Variance between locations was homogeneous only for four of the eight bulb traits (C/B, RT, SSC, and PA). Because of this, combining ability data will be presented for both individual environments and in a combined analysis.

The genotype mean squares were significant for all traits except SSC and PA in Uvalde; SSC in La Mesa; and R/B, SSC, and PA in the combined analysis. The only significant rep mean square was R/B in Uvalde. Analyses for combining ability were conducted to partition the genotypic variation into GCA, SCA, REC, MAT, and NMAT effects (Tables 8, 9, and 10). The GCA effect was significant for all traits except R/B, SSC, and PA in Uvalde; BD and SSC in La Mesa; and R/B, SSC, and PA in the combined analysis. The SCA effect was significant for all traits except RT, SSC, and PA in Uvalde; BH, C/B, RT, and SSC in La Mesa; and R/B, SSC, and PA in the combined analysis. The reciprocal effect was significant only for BD, RT, and BW in Uvalde; C/B and RT in La Mesa; and BH, R/B, SSC, and PA in the combined analysis. The maternal effect was significant for BD, RT, and BW in Uvalde; C/B and RT in La Mesa; and C/B and RT in the combined analysis. The nonmaternal effect was significant only for BD in Uvalde; RT in La Mesa; and BD, RT, and BW in the combined analysis. In the combined analysis, the environmental effect was only significant for C/B and none of the environmental interactions were significant.

To determine the ability of breeders to predict hybrid performance, the importance of GCA and SCA was measured for each trait (Table 11). The closer the calculated values are to unity, the better the predictability based on GCA. Similar results

were obtained in the two locations except for with R/B. The combined analysis generally gave an estimate equal to the average of the two environmental estimates except for with SSC. Traits with high predictability based on GCA included BH, C/B, RT, and PA.

Although there were differences in which combining ability effects were significant between the two locations and the combined analysis, the direction of significant effects was the same for all but one trait (Tables 12 through 19). The exception to this was R/B, which had one difference in sign for GCA, SCA, and MAT.

Table 8. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown in Uvalde, TX.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Reps	2	0.06	0.01	0.07	1.06***	0.0011	3374.50	0.21	0.30
Genotype	15	0.47***	0.10***	0.96***	0.77***	0.0030***	14954.43***	1.73	1.26
GCA	3	0.35**	0.30***	2.25***	0.28	0.0115***	18518.56***	0.59	2.62
SCA	6	0.78***	0.08***	0.98***	1.23***	0.0006	22230.86***	0.72	0.71
REC	6	0.21**	0.01	0.30	0.55***	0.0010	5895.93*	3.32	1.14
MAT	3	0.27**	0.01	0.52*	0.96***	0.0012	7180.85*	2.59	1.66
NMAT	3	0.16*	0.00	0.08	0.14	0.0009	4611.01	4.04	0.62
Error	30	0.05	0.01	0.17	0.10	0.0005	1998.77	1.52	0.78
R^2		0.82	0.78	0.74	0.82	0.75	0.79	0.37	0.45
% CV		7.11	5.59	24.38	4.23	12.06	20.65	22.22	22.41

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 9. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown in La Mesa, NM.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Reps	2	0.01	0.03	0.61	0.39	0.0004	203.85	1.18	1.57
Genotype	15	0.35*	0.13***	0.93**	1.09*	0.0034***	13556.03***	0.93	15.04***
GCA	3	0.27	0.47***	2.22***	2.35**	0.0065***	16647.22**	0.48	57.35***
SCA	6	0.58**	0.06	0.46	1.10	0.0020*	18833.60***	1.19	6.66*
REC	6	0.16	0.02	0.76*	0.46	0.0032**	6732.86	0.92	2.14
MAT	3	0.06	0.01	1.45**	0.70	0.0040**	3789.63	0.28	0.98
NMAT	3	0.26	0.03	0.07	0.21	0.0025*	9676.09	1.56	2.87
Error	30	0.13	0.03	0.29	0.47	0.0008	3472.93	1.28	2.11
R^2		0.57	0.67	0.64	0.55	0.69	0.66	0.31	0.79
% CV		10.86	8.61	24.77	9.61	14.81	25.26	26.28	26.32

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 10. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown in Uvalde, TX and La Mesa, NM.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Environment	1	0.29	0.02	5.96***	3.71	0.0004	6758.82	37.11	57.03
Reps (Env)	4	0.03	0.07	0.34	0.72	0.0007	1789.18	0.69	0.94
Genotype	15	0.71***	2.99***	1.65***	1.14	0.0056***	26218.25***	0.92	10.06
GCA	3	0.56**	0.73***	4.20***	1.19	0.0172***	32940.55***	0.58	35.26
SCA	6	1.24***	0.12***	1.14***	2.04	0.0016*	38828.80***	0.62	5.18
REC	6	0.26*	0.02	0.89**	0.21	0.0038***	10246.54**	1.43	2.35
MAT	3	0.12	0.01	1.73***	0.34	0.0046***	7115.00	0.73	2.04
NMAT	3	0.40**	0.03	0.05	0.07	0.0029**	13378.08**	2.16	2.48
G X E	15	0.10	0.35	0.24	0.72	0.0007	2292.21	1.72	6.25
GCA X E	3	0.06	0.04	0.27	1.44	0.0008	2225.23	0.48	24.70
SCA X E	6	0.12	0.02	0.29	0.28	0.0010	2235.65	1.29	2.19
REC X E	6	0.11	0.01	0.17	0.80	0.0005	2382.25	2.81	0.93
MAT X E	3	0.20	0.02	0.24	1.32	0.0005	3855.48	2.14	0.59
NMAT X E	3	0.02	0.01	0.10	0.28	0.0005	909.02	3.44	1.00
Error	60	0.09	0.02	0.23	0.28	0.0006	2735.85	1.40	1.43
R^2		0.69	0.71	0.72	0.67	0.72	0.73	0.49	0.78
% CV		9.24	7.28	24.83	7.29	13.48	23.26	24.00	25.34

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 11. The relative importance of general combining ability (GCA) and specific combining ability (SCA) in determining hybrid performance for a given bulb trait.

	Bulb Trait[z]							
Location[x]	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Uvalde	0.47[y]	0.88	0.82	0.32	0.97	0.62	0.62	0.88
La Mesa	0.49	0.94	0.91	0.81	0.87	0.64	0.44	0.95
Combined	0.48	0.93	0.88	0.54	0.96	0.63	0.65	0.93

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
[x]TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
[y]Values calculated using GCA and SCA mean squares (g_i and s_i respectively) from the analyses of variance using the formula $2\ g_i /(2\ g_i + s_i)$.

Table 12. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb diameter.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	-0.12**	-0.03										
	2	-0.09*	-0.10*	1 x 1	-0.04	-0.34*	-0.19*						
	3	0.11**	-0.02	1 x 2	0.15	0.33**	0.24**	0.06	-0.03	0.02	-0.02	0.07	0.03
	4	0.10*	0.15***	1 x 3	0.06	0.1	0.08	-0.04	-0.08	-0.06	-0.02	-0.08	-0.05
				1 x 4	-0.12	0.25	0.07	-0.13	-0.02	-0.08	0.04	0.01	0.03
NM	1	-0.04	-0.03	2 x 2	-0.23*	-0.13	-0.18						
	2	-0.12	0.07	2 x 3	-0.28***	-0.24*	-0.26***	-0.25*	-0.04	-0.14	-0.16*	-0.14	-0.15*
	3	0.03	-0.04	2 x 4	0.60***	0.18	0.39**	-0.11	0.28	0.08	0.14*	0.21*	0.18**
	4	0.13*	0	3 x 3	-0.25*	-0.3	-0.28**						
				3 x 4	0.73***	0.75**	0.74***	-0.35***	-0.27	-0.31***	-0.18**	-0.23*	-0.20**
Comb	1	-0.08*	-0.03	4 x 4	-1.20***	-1.18**	-1.19***						
	2	-0.10**	-0.02										
	3	0.07	-0.03										
	4	0.11**	0.08										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 13. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb height.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.01	-0.01										
	2	-0.06*	-0.01	1 x 1	0.03	-0.07	-0.02						
	3	-0.11***	-0.02	1 x 2	0.02	0.14*	0.08*	0.01	0.02	0.01	0	0.05	0.02
	4	0.15***	0.04	1 x 3	0	-0.04	-0.02	0.02	-0.04	-0.01	0.01	-0.05	-0.02
				1 x 4	-0.09	0.04	-0.03	-0.05	0.02	-0.02	-0.01	0	0
NM	1	0.10**	0	2 x 2	-0.03	-0.04	-0.03						
	2	-0.09**	0.03	2 x 3	-0.12**	-0.09	-0.10**	-0.03	0.01	-0.01	-0.03	-0.02	-0.03
	3	-0.15***	-0.01	2 x 4	0.14	0.02	0.08	-0.02	0.12	0.05	0.03	0.07	0.05
	4	0.14***	-0.02	3 x 3	-0.04	-0.02	-0.03						
				3 x 4	0.20*	0.16	0.18**	-0.08	-0.06	-0.07	-0.02	-0.07	-0.05
Comb	1	0.06**	0	4 x 4	-0.26*	-0.22	-0.24*						
	2	-0.07**	0.01										
	3	-0.13***	-0.01										
	4	0.14***	0.01										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 14. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for number of centers per bulb.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	-0.37***	-0.12										
	2	0.07	0.01	1 x 1	0.14	0.03	0.09						
	3	-0.06	-0.09	1 x 2	0.07	0.3	0.19	-0.01	-0.54*	-0.27	0.13	-0.06	0.03
	4	0.36***	0.20**	1 x 3	-0.05	-0.22	-0.13	-0.05	-0.22	-0.13	-0.02	0.03	0.01
				1 x 4	-0.3	-0.15	-0.23	-0.43*	-0.52*	-0.48**	-0.11	0.03	-0.04
NM	1	-0.40***	-0.32**	2 x 2	-0.58**	-0.50*	-0.54**						
	2	0.27**	0.16	2 x 3	-0.13	-0.1	-0.12	0.15	0.1	0.13	0.04	-0.13	-0.04
	3	-0.07	-0.07	2 x 4	1.22***	0.80*	1.01***	-0.1	-0.01	-0.05	0.09	0.06	0.08
	4	0.20*	0.23*	3 x 3	-0.08	0.37	0.15						
				3 x 4	0.33	-0.41	-0.04	-0.27	-0.39	-0.33*	0.02	-0.09	-0.03
Comb	1	-0.39***	-0.22**	4 x 4	-1.25**	-0.24	-0.74						
	2	0.17*	0.09										
	3	-0.07	-0.08										
	4	0.28***	0.21**										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 15. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for number of rings per bulb.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	-0.02	0.16*										
	2	0.1	-0.19**	1 x 1	-0.18	-0.59	-0.38						
	3	-0.15*	-0.16*	1 x 2	0.24*	0.13	0.18	0.47**	-0.62*	-0.08	0.11	-0.23	-0.06
	4	0.07	0.18**	1 x 3	0.18	0.42	0.3	0.21	-0.18	0.01	-0.11	0.1	-0.01
				1 x 4	-0.07	0.62	0.28	-0.02	-0.2	-0.11	0	0.12	0.06
NM	1	-0.40**	-0.25*	2 x 2	-0.38*	0.19	-0.1						
	2	0.22	0.14	2 x 3	-0.44***	-0.52*	-0.48	-0.07	0	-0.03	-0.03	-0.1	-0.07
	3	0.27*	0.04	2 x 4	0.96***	0.01	0.48	-0.22	-0.05	-0.14	0.15	-0.12	0.01
	4	-0.1	0.07	3 x 3	-0.16	-0.3	-0.23						
				3 x 4	0.57*	0.7	0.63	-0.49**	-0.03	-0.26	-0.15	0.00	-0.07
Comb	1	-0.21	-0.04	4 x 4	-1.46***	-1.33	-1.4						
	2	0.16	-0.02										
	3	0.06	-0.06										
	4	-0.02	0.12										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 16. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for ring thickness.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.012*	0.006	1 x 1	-0.004	-0.034**	-0.019*						
	2	-0.013**	-0.006	1 x 2	-0.002	0.013	0.006	0	0.012	0.006	-0.013	-0.01	-0.011*
	3	0.025***	0.006	1 x 3	0.008	0.01	0.009	0.002	-0.005	-0.002	0.001	-0.012	-0.005
	4	-0.023***	-0.006	1 x 4	0.001	0.044*	0.023	0.023*	0.050***	0.037***	0.011	0.022**	0.017**
NM	1	0.015**	0.014**	2 x 2	0.013	-0.004	0.005						
	2	-0.013*	-0.008	2 x 3	0.003	-0.003	0	-0.022*	-0.023*	-0.023**	-0.01	-0.009	-0.009
	3	0.013*	0.007	2 x 4	-0.027	-0.003	-0.015	-0.003	0.005	0.001	-0.003	-0.001	-0.002
	4	-0.015**	-0.014**	3 x 3	-0.016	-0.026*	-0.021**						
				3 x 4	0.021	0.045*	0.033**	0.003	0	0.002	-0.008	-0.021*	-0.015**
Comb	1	0.013***	0.010**	4 x 4	0.005	-0.086**	-0.040*						
	2	-0.013***	-0.007*										
	3	0.019***	0.006*										
	4	-0.019***	-0.010**										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 17. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb weight.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	-22.5**	-3.8										
	2	-20.2*	-13.8	1 x 1	-5.6	-39.4	-22.5						
	3	5.8	-7.6	1 x 2	19.4	51.6	35.5**	4.8	-7.4	-1.4	-5.3	9.3	2.0
	4	37.0***	25.2**	1 x 3	1.9	7.4	4.6	-4.9	-6.0	-5.4	-8.7	-16.0	-12.4
				1 x 4	-10.0	19.9	5.0	-15.0	-7.5	-11.3	14.0	6.8	10.4
NM	1	-5.4	-5.2	2 x 2	-30.5	-27.8	-29.1						
	2	-22.4*	11.5	2 x 3	-46.1**	-39.2*	-42.7***	-30.3	-2.8	-16.6	-24.1	-29.5	-26.8*
	3	-10.3	-15.3	2 x 4	87.7**	43.2	65.5**	-20.2	41.3	10.5	18.8	38.8*	28.8**
	4	38.0***	9.0	3 x 3	-39.7*	-56.0*	-47.9**						
				3 x 4	123.7***	143.9***	133.8***	-65.5***	-69.8**	-67.7***	-32.8*	-45.5*	-39.2***
Comb	1	-14.0*	-4.5	4 x 4	-201.5***	-207.0**	-204.2***						
	2	-21.3**	-1.2										
	3	-2.2	-11.4										
	4	37.5***	17.1**										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 18. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for soluble solids content.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	-0.04	0.14										
	2	-0.21	-0.11	1 x 1	0.28	-0.13	0.03						
	3	0.14	0.37	1 x 2	-0.17	0.66	0.23	0.01	0.5	0.25	-0.24	0.45	0.14
	4	0.11	-0.4	1 x 3	-0.32	-0.3	-0.24	-0.94	0.14	-0.52	-0.7	-0.03	-0.43
				1 x 4	-0.07	-0.11	-0.06	1.47	-0.52	0.48	0.94	-0.42	0.29
NM	1	0.14	0.03	2 x 2	0.69	-0.65	0.03						
	2	-0.08	-0.02	2 x 3	-0.14	-0.22	-0.2	-0.14	0.59	0.23	0.34	0.46	0.43
	3	-0.16	-0.14	2 x 4	-1.08	0.87	-0.1	-0.3	-0.15	-0.22	-0.58	-0.01	-0.29
	4	0.1	0.13	3 x 3	0.18	0.06	0.08						
				3 x 4	0.09	0.41	0.28	0.41	0.17	0.29	-0.36	0.43	0.01
Comb	1	0.06	0.05	4 x 4	1.06	-1.17	-0.11						
	2	-0.16	-0.06										
	3	0	0.14										
	4	0.09	-0.14										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 19. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for pyruvic acid content.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.38	0.29										
	2	0.05	0.05	1 x 1	-0.06	0.95	0.47						
	3	-0.01	0.01	1 x 2	0.36	-0.57	-0.1	0.05	0.54	0.29	-0.18	0.31	0.04
	4	-0.42	-0.35	1 x 3	-0.19	-0.78	-0.53	0.07	-0.35	-0.07	-0.2	-0.87	-0.5
				1 x 4	-0.05	-0.55	-0.32	1.03	0.8	0.91	0.39	0.56	0.46
NM	1	-0.49	0.25	2 x 2	-0.18	0.52	0.16						
	2	1.86***	0.02	2 x 3	0.31	1.37**	0.85	0.1	0.82	0.46	0.05	0.53	0.27
	3	0.45	-0.28	2 x 4	-0.31	-1.84	-1.08	0.17	-0.22	-0.02	-0.24	-0.22	-0.23
	4	-1.81***	0.01	3 x 3	-0.12	-0.01	-0.04						
				3 x 4	0.13	-0.58	-0.24	0.21	-0.63	-0.21	-0.15	-0.34	-0.23
Comb	1	-0.06	0.28	4 x 4	0.24	2.98*	1.64						
	2	0.96	0.04										
	3	0.21	-0.15										
	4	-1.11	-0.17										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

DISCUSSION

Crosses

The reduction of the diallel from seven to four parents severely limits the amount of useful information that can be gleaned from this experiment. Seven parents, although less than the 10 recommended by some authors (Hayman, 1960), is within the range seen in the literature (Christie and Shattuck, 1992). Although it would also have been analyzed as a completely fixed model, the resulting combining ability estimates would have been based on the entire DH group to date at Texas A&M University. The current experiment is only representative of just over half of the group. As a result, the combining abilities obtained can only be used as a preliminary guide to determine appropriate breeding and selection strategies for this material.

Variation

There were several factors which contributed to increased variation in the experiment. The first factor was a last-minute change in location which resulted in the transplants going to La Mesa spending an extra month in the flats. It is unknown what effect this extra time in the flats would have on the bulb traits measured. The location change also resulted in differences in how the plots were set up (80" beds vs. every other bed on 40" beds) and irrigated (drip vs. furrow). Any variance between locations, caused by the difference of time in transplant trays and the differences in plot set-up, would be separated out in the ANOVA and become part of the environmental component.

The second factor was damage to transplants that occurred during transport to Uvalde. The flats, containing all of the transplants for both locations, collapsed on top of each other causing significant damage to the onion plants. Although there was a difference in the extent of the damage on each flat, it was not possible to quantify this difference. The damage resulted in uneven stands in Uvalde, due to the death of some plants, and a reduction in the number of plants per plot in both locations. This factor would have increased within plot variation and could also have contributed to some variation between certain plots.

The third factor was a problem with weeds and harvester ants in Uvalde. The weeds were a result of midseason rains which prevented access to the plots for weed removal. During this time, harvester ants came in from an adjacent uncultivated field and cut off leaves and some times entire plants from areas near their tunnels. The number of weeds growing tended to increase in a direction parallel with the blocks. That means that its effects would likely be included in block variance. It would only increase variance between plots if the entries responded differently to the increased competition. The randomness of the ant damage would probably result in its effects being contributed to experimental error.

The fourth factor was the weather during the growing season in La Mesa. The weather was cooler than average for most of the growing season, with a sudden spike in temperature towards the end of the season. This dramatic shift in temperature occurred almost one month prior to harvest, resulting in the rapid maturity of the onions. Because the later maturing crosses were not very far into the bulb expansion phase of their

growth cycle, they were likely affected more than early maturing crosses. This likely contributed to variation in yield between the two locations, which would be separated out in the ANOVA and become part of the environmental component.

The fifth factor was the nature of the crosses. Because no male sterility was available in these DH lines, all crosses performed were fertile by fertile crosses. As a result, the seed produced by the female plant in each cross contained some seed that was the result of self pollination. In the crosses in which the two parents were different colors, the selfed individuals are easy to distinguish and discard. However, in crosses involving parents of the same color, it was not possible to separate out these individuals. The amount of self pollination in the different colored crosses was calculated for each cross. For all crosses included in the field experiment the range of self pollination was from 24% to 96%, with an average of 67% overall. For the crosses included in the diallel the range of self pollination was 27% to 85%, with an average of 66% overall. The inclusion of selfed progeny with the hybrids would tend to increase the within variance in these plots and would make the entry appear more like the female parent than it really is, resulting in an inflated maternal effect.

Mean Performance

The hybrids between the DH lines generally showed good vigor, with many of the crosses giving larger BD and BW than the commercial checks. Unfortunately, these vigorous bulbs also had significantly greater C/B than the commercial checks. Almost all of the hybrids were flatter than the commercial checks, a trait inherited from their parents. Although the number of centers per bulb exceeded commercially acceptable

levels in many of the crosses, it must be realized that most of the DH lines also displayed this trait. It may be possible to reduce this problem by increasing selection intensity on parental material before creating DH lines.

Combining Ability

The absence of significant environmental variance components (except for C/B), and combining ability effect by environmental variance components, implies that duplicate trials in separate locations or years would not be necessary with these lines. This is valuable information for a breeding program because it saves both time and money. However, further testing with additional lines is required to see if this decreased sensitivity to environmental factors is characteristic of DH lines, or if it is only true of the lines included in this experiment.

Apparent trends existed between the GCA effects of a line in the different bulb traits. For the most part, lines that tended to increase BD in their progeny also increased BH, C/B, BW, and either R/B or RT. This relationship is not surprising, but shows the inappropriateness of using these lines as parents to create hybrids: the goal of onion breeding is to reduce C/B while increasing traits related to bulb size. In all but one case, the effects of R/B and RT were opposite in their direction. The exception, line H8 in La Mesa, tended to increase both R/B and RT in its progeny. However, the trend did not occur at both locations. This would be a valuable trait in a parent if it held true because it should also result in increased bulb size.

The ability to predict the performance of a hybrid by the general combining ability of its parents would be very valuable to a plant breeder. This ability would allow

the breeder to determine which hybrid crosses to make without extensive testing. Also, the breeder could cross the best combiners to create improved breeding populations from which to select new parents. For the lines in this study, prediction of hybrid BH, C/B, RT, and PA should be quite reliable. For C/B, the value was high for La Mesa but not for Uvalde. This was the one trait for which additional locations would be necessary to determine hybrid performance.

Implications for breeding

To be a useful parent in a breeding program, a line must have good mean performance as well as good combining ability. Some of the lines used in this study matched this requirement for some of the traits. For example, H11 (parent 4) was comparable to the commercial checks for BH and also had significant positive GCA in both locations. In breeding, however, being good for one trait is not sufficient. A line must have good performance and good combining ability for multiple traits.

The lines and hybrids in this study had the most problems with C/B. This trait is very important in the industry. Although it is beneficial to find out which of the DH lines are good combiners, the C/B observed in the F_1s of the best combiners would not be commercially acceptable. In addition, the combining abilities are relative to the parents used and therefore only compare to the other lines in the study. Because only four of the seven DH lines were included in the diallel, it is not known how the other three lines would perform. It is also unknown how these DH lines perform compared to conventional breeding lines.

Further testing is needed of the other three DH lines to see if they might produce hybrids with increased vigor, but without the associated increase in C/B. Even if these DH lines are not able to be used directly as parents, they could still be useful in a breeding program. They, and other DH lines created for this purpose, could be used as part of a long-term program to reduce inbreeding depression in onion by going through several cycles of recurrent selection similar to what was done with inbred lines in corn.

CHAPTER V

DOUBLED HAPLOID BY CONVENTIONAL INBRED DIALLEL

INTRODUCTION

Onion doubled haploid (DH) lines were first reported in the literature by Muren (1989). The creation of completely homozygous lines is something of a paradox due to the severe inbreeding depression in onion. Although it is tempting to speculate that the technique provides selection against inbreeding depression, a second cycle of gynogenesis in onion showed considerable increase in only one of three lines tested (Javornik et al., 1998). Whether this process selects against deleterious recessive genes, or is simply selecting for lines which have the ability to regenerate in vitro, the existence of homozygous lines in onion could be a valuable tool for studying their usefulness in breeding and genetics.

The diallel mating design devised by Griffing (1956) is a valuable tool for breeders. It allows the partitioning of the variance among crosses into genetic and environmental components. The variation due to genetic components can be further subdivided into main effects (general and specific combining abilities) and interactions (reciprocal effects). The general and specific combining abilities are related to additive and dominant gene action, respectively. The reciprocal effects can be further subdivided into maternal and nonmaternal effects, as described by Cockerham (1963). Baker (1978) concluded that diallels should only be used to estimate combining ability effects, citing the difficulty of meeting the assumptions of independent distribution of genes in the

parents and no epistasis. Christie and Shattuck (1992) stated that the estimation of combining ability effects requires no assumptions about a reference population. This makes it valuable for crops in which it is difficult to create genetic populations for study. The knowledge of combining ability effects allows breeders to determine the best breeding and selection strategies to use in trait improvement.

General combining ability predominates for all traits reported in onion (Hosfield et al., 1976; Hosfield et al., 1977a; Krueger et al., 1989). Reciprocal effects, although not very common in plants (Hosfield et al., 1976), have been significant for several traits in each of the above-mentioned reports on onion. Hosfield et al. (1977a) found that fewer traits showed significant reciprocal effects after extending their study over two years. They attributed this to overestimation of the effects in their first experiment, although they could not eliminate bias due to different numbers of parents in the two experiments. However, even in their second experiment three out of eight traits retained significant reciprocal effects. It is not known exactly why reciprocal effects continue to be important in onion; however, this demonstrates the importance of choosing experimental designs capable of measuring these effects.

Information is available on heterosis estimates in onion. Initial high parent heterosis (HPH) estimates for yield in onion ranged from -26 to 192% (Jones and Davis, 1944). However, with the subsequent improvement of inbreds, heterosis estimates have been depressed. This is because part of the heterosis observed when inbred parents are crossed is due to the recovery of lost vigor due to inbreeding depression. More recent

studies give HPH estimates for yield of 12 to 20 % for long day inbreds and ≤ 50 % with short day inbreds (Hosfield et al., 1977b; Netrapal and Singh, 1999).

In hybrid development, onion breeders strive to maximize uniformity while maintaining a certain level of vigor in the inbred parents. Because of severe inbreeding depression, parents used to create hybrids are not true inbreds; usually a maximum of three selfing cycles are possible, followed by recurrent selection (Pike, 1986). After desirable parents are identified, male sterile pairs are created for inbred parents by backcrossing. Remnant heterogeneity and heterozygosity in the male sterile parent and remnant heterozygosity in the male fertile parent would result in depressed heterosis estimates. The only way to obtain unbiased estimates is to use homozygous parent lines.

The objectives of this experiment are 1) to compare the mean performance and combining ability effects of DH lines to equivalent conventional inbred lines, 2) to compare mean performance and heterosis estimates of DH by DH crosses to those of DH by conventional inbred crosses.

MATERIALS AND METHODS

Plant Material

Seven DH lines were developed at Texas A&M University from five different short-day F_2 and F_2M populations following the procedure described by Kim et al. (2004). The original parental material of the DH lines are ‘1015’ (US open-pollinated variety), ‘1025’ (US open-pollinated variety), ‘Ori’ (Israeli open-pollinated variety), ‘Ringer’ (US open-pollinated variety), and ‘Cardinal’ (US hybrid). Pedigrees are as

follows: ‘Ori’ X ‘Ringer’ (H1), ‘Ringer’ X ‘Ori’ (H6, H8), ’1015’ X ‘Ori’ (H2, H7), ‘Ori’ X ‘Cardinal’ (H4), and ‘1025’ X ‘Ringer’ (H11). Although two pairs of DH lines (H2, H7; and H6, H8) came from the same mother plant, they are phenotypically distinct from one another in both cases.

Four conventional inbred lines selected from the same parental crosses as the DH lines were identified in the breeding program. Each conventional inbred line was given a designation to show its relationship to the DH line. For example, the conventional line descended from the ‘Ori’ X ‘Ringer’ cross was given the designation I1, 6, 8 to show its relationship to H1, H6, and H8. The generation of the conventional inbreds used is I1, 6, 8 (F_2M_3); I2, 7 (F_2M_3); I4 (F_3); and I11 (BC_4). Lines I11 had an A-line pair that was being developed in the breeding program: this line (SM_4) was designated I11A. Each of these designations for generation are those commonly used in breeding with F referring to filial generation, M referring to mass, S referring to self, and BC referring to backcross.

Crosses

A complete diallel crossing scheme involving the seven DH lines and four of the five conventional inbreds, no reciprocal crosses could be made for the male sterile line I11A, was attempted as described by Griffing (1956). However, not all of the crosses could be made because of unusually warm winter weather which reduced vernalization and the resultant flowering of the parent plants. Attempts were made in two successive years to vernalize non-bolting onion plants. The first year, non-bolting plants were placed in a cold room (40 ºC) for two to four weeks. Although some bolting did occur,

the scapes died shortly after emergence from the bulb. The second year, non-bolting onions were harvested and the bulbs were cut in half and grown in a growth chamber. Unfortunately, the onions had to be removed from the growth chamber shortly after new leaves emerged from the bulbs and the young plants died shortly after being transferred to the greenhouse. The failure to induce bolting in many of these lines resulted in crosses being made with only a few plants per line. As a result, a complete diallel was only obtained for four lines (H1; H11; I2, 7; and I11). Crosses made to I11A were also included to determine if the substitution of these crosses for same color crosses to I11 would be appropriate. The goal was the elimination of selfs among the hybrids in same color crosses made to I11.

Experimental Design

Seeds from the crosses (Table 20) were sown in 288-well transplant trays (Dillen Products Inc., USA) on November 6th and grown in the greenhouse until transplanting. Transplants were arranged in an α-lattice incomplete block design (Patterson and Williams, 1976) with three reps per entry. Plots were ten feet long with two foot alleys. Two rows, twelve inches apart, were planted in each plot with plants spaced five inches apart.

The trial in Uvalde, TX was transplanted on January 14th and 15th. Plots were placed in rows eighty inches apart on low pressure drip tape (Netafim USA, USA). The trial in La Mesa, NM was planted on February 16th and 17th under furrow irrigation.

Because rows in the field were forty inches apart, experimental plots were planted on every other row. A single row of Texas Early White was planted in the rows between plots.

Table 20. List of crosses made between two doubled haploid lines (H), two conventional lines (I), and one male sterile line (I11A).

♀ ♂	H1	H11	I2, I7	I11	I11A
H1	1	6	11	16	20
H11	2	7	12	17	21
I2,I7	3	8	13	18	22
I11	4	9	14		23
I11A	5	10	15	19	

Phenotypic Evaluation

Plots were harvested at each location at maturity (>80% tops down). Up to ten bulbs (see appendix) were selected from each plot and were measured for eight traits: bulb diameter (BD), bulb height (BH), number of centers per bulb (C/B), number of rings per bulb (R/B), ring thickness (RT), bulb weight (BW), soluble solids content (SSC), and pyruvic acid content (PA). Measurement for BD was taken at the bulbs widest point; if the bulb was an obvious double, an average of two measurements taken on opposite sides was used. The measurement for BH was taken immediately adjacent to the root mass at the base and the dried leaves at the top of the bulb. For C/B, bulbs with all growing points located within a center diameter of 3.0 cm were considered single center. If the bulb did not meet this standard, the total number of growing points was tallied. RB was a measurement of rings that continued at least half way around the

bulb. RT was measured on the second ring of the bulb. If the ring varied in thickness, the measurement was taken at a location that was considered representative of the average ring thickness for the bulb. Bulbs were weighed individually to obtain BW. SSC and PA were measured from frozen juice as described below.

Bulbs were cut horizontally into two halves, with a 5 mm slice removed from the top half. The slice was placed in a plastic baggie and crushed with a hand press. The sample was then left at room temperature for 20-30 minutes and then placed in a freezer at -20 ºC. Juice from all samples was analyzed for soluble solids content using a hand refractometer (10430; American Optical Corp., Buffalo, NY, USA) and pyruvic acid content was measured according to Schwimmer and Weston (1961) as modified by Yoo and Pike (1999).

Statistical Analysis

Individual plant data were used to calculate mean performance of the five parent lines, the 10 hybrid crosses and their reciprocals, and two commercial checks; 'Legend', one experimental hybrid made with 'Legend'. The data were analyzed in SAS 9.1.3® using proc glm and proc mixed. Efficiency (e) of the design was determined by the formula:

$$e = \left(\frac{SED_{RBD}}{SED_{IB}} \right)^2$$

where

SED_{RBD} is the standard error of the difference of means using the RBD design;

SED_{IB} is the standard error of the difference of means using the IB design

The adjusted means from the design that gave the lowest SED values were used to determine mean performance and to compare to the commercial checks.

The combining ability analyses were conducted with plot averages from all possible crosses between four parental lines (H1; H11; I2, 7; and I11) designated as parents 1 through 4 respectively. The data were analyzed as an all fixed model (Model 1 Method 1) as described by Griffing (1956) using the program Diallel-SAS05 (Zhang et al., 2005). The program analyzes the data using unadjusted means with a RCB design. The data were analyzed in each individual environment and in a combined environment analysis. Homogeneity of variance tests between the two environments were conducted as described by Snedecor and Cochran (1967). To determine the relative importance of GCA and SCA in predicting hybrid performance for these traits, the following formula was used (Baker, 1978):

$$\frac{2g_i}{2g_i + s_{ij}}$$

where

g_i is the mean square of the GCA effect in the combining ability ANOVA;

s_{ij} is the mean square of the SCA effect in the combining ability ANOVA

RESULTS

Locations

Trait means between the two locations were comparable for the most part (Table 21). Trait means were higher in Uvalde than in La Mesa except for RB, RT, and SSC. The variance was generally greater in La Mesa.

Table 21. Means and $LSD_{0.05}$ for bulb traits at two locations.

	Uvalde, TX		La Mesa, NM	
Character	**General Mean**	**LSD**	**General Mean**	**LSD**
Diameter (in.)	3.54	0.35	3.14	0.36
Height (in.)	2.38	0.19	2.21	0.20
Centers/bulb	2.44	0.46	1.87	0.47
Rings/bulb	7.00	0.65	7.00	0.72
Ring thickness (in.)	0.176	0.022	0.183	0.030
Bulb weight (g)	302.38	70.31	223.21	62.69
Soluble Solids (brix)	4.12	0.87	5.63	1.13
Pungency (µM pyruvate/ml)	4.30	0.83	4.20	1.23

Mean Performance

Efficiency values were consistent between traits. However, in La Mesa the randomized complete block (RCB) design used by proc glm did a better job of partitioning variance, thus reducing the efficiency values to about half of what they were in Uvalde. The incomplete block (IB) design used by proc mixed was more efficient at partitioning the variance, as evidenced by the lower SED values obtained and the efficiency values greater than unity, than the RCB design for all traits tested (Table 22).

Table 22. Comparison of SED values given by analysis as a randomized complete block (RCB) and an α-lattice (IB).

	Uvalde, TX			La Mesa, NM		
Character	**RCB**	**IB**	**Efficiency**	**RCB**	**IB**	**Efficiency**
Diameter (in.)	0.61	0.18	41.92	0.50	0.18	20.23
Height (in.)	0.34	0.10	41.83	0.28	0.10	22.12
Centers/bulb	0.78	0.24	36.04	0.64	0.24	19.59
Rings/bulb	1.10	0.33	35.68	1.00	0.37	20.07
Ring thickness (in.)	0.04	0.01	41.81	0.04	0.02	22.25
Bulb weight (g)	124.19	35.87	41.49	88.36	31.98	21.08
Soluble Solids (brix)	1.47	0.45	35.67	1.54	0.58	18.99
Pungency (μM pyruvate/ml)	1.40	0.42	35.93	1.85	0.63	25.54

[z]Measured as $e=(SED_{RCB}/SED_{IB})^2$ where the numerator and denominator are the standard error of the difference of means using the RCB and IB designs respectively.

The mean performance of the parent lines (1, 7, and 13) and selfs (20, 21, 22, and 23) was generally less than the commercial checks, except for with C/B and PA (Tables 23, 24). Parent lines generally performed similarly in the two environments, with respect to the commercial checks, except with BD, C/B, and BW. In La Mesa, all three had considerably lower BD, one had lower C/B (1), and one had lower BW (13). The progeny from the selfing of the line performed similarly in the two environments, with respect to the commercial checks, except with SSC. In La Mesa, three lines had higher SSC (20, 22, and 23).

Most of the hybrids were statistically similar to the commercial checks for the traits measured, except for BH, C/B, and R/B (Tables 23, 24). About half of the hybrids had lower BH, most had higher C/B, and about half had lower R/B than the commercial checks. The hybrids performed similarly in the two environments, relative to the commercial checks, except for SSC and PA. In La Mesa, approximately one third of the hybrids had higher SSC. In Uvalde, about two thirds of the hybrids had higher PA.

Combining Ability

Variance between locations was homogeneous only for three of the eight bulb traits (C/B, R/B, and SSC). Because of this, combining ability data will be presented for both individual environments and in a combined analysis.

In the combined analysis, there was a significant environmental effect for BD, BH, C/B, and BW. However, there were no significant rep effects in any of the analyses. The genotype mean squares were significant for all traits except SSC in Uvalde, La Mesa, and in the combined analysis. Analyses for combining ability were conducted to partition the genotypic variation into GCA, SCA, REC, MAT, and NMAT effects (Tables 25, 26, and 27). The GCA effect was significant for all traits except BW and SSC in both single environment analyses and SSC in the combined analysis. The SCA effect was significant for all traits except RT and SSC, in Uvalde; BD, BH, and RT in La Mesa; and SSC in the combined analysis. The reciprocal effect was significant only for BH and C/B in Uvalde; and BD, BH, C/B, and BW in the combined analysis.

Table 23. Mean performance of entries at Uvalde, TX.

Entry	Parent 1	Parent 2	Bulb Trait[z]							
			BD	BH	C/B	R/B	RT	BW	SSC	PA
1	1	1	3.31	1.89	2.10	6.72	0.21	208.37	3.84	7.06
2	2	1	4.14	2.43	3.33	7.73	0.18	431.48	4.48	5.34
3	3	1	3.98	2.57	2.18	7.77	0.21	384.80	3.73	5.27
4	4	1	4.02	2.38	2.75	7.28	0.21	420.05	3.88	4.90
5	5	1	4.21	2.49	3.23	8.06	0.17	430.06	5.34	4.65
6	1	2	3.85	2.36	3.52	7.75	0.18	354.37	2.00	5.13
7	2	2	3.07	2.17	2.43	6.29	0.16	202.70	4.26	3.06
8	3	2	2.99	2.33	1.97	6.48	0.17	219.18	4.73	3.97
9	4	2	3.36	2.24	2.73	5.96	0.16	240.81	3.79	3.13
10	5	2	3.20	2.29	2.45	6.33	0.15	239.94	5.01	3.65
11	1	3	4.01	2.59	2.46	7.76	0.21	434.48	2.98	4.45
12	2	3	3.25	2.38	2.23	6.38	0.18	286.53	4.11	3.18
13	3	3	3.20	2.58	1.51	7.23	0.18	260.02	4.74	6.37
14	4	3	3.63	2.28	2.81	6.74	0.17	288.68	4.31	3.13
15	5	3	3.58	2.58	2.65	7.06	0.19	304.68	5.38	4.05
16	1	4	4.01	2.36	2.69	7.54	0.20	371.67	4.22	5.47
17	2	4	3.39	2.30	2.96	6.29	0.14	254.44	3.87	2.63
18	3	4	3.55	2.63	2.38	7.23	0.18	342.02	4.02	4.41
19	5	4	3.87	2.52	3.21	6.69	0.16	364.49	3.36	2.52
20	1	self	3.21	1.88	1.85	7.99	0.17	191.74	4.46	7.50
21	2	self	3.21	2.22	2.92	6.62	0.12	217.48	4.86	3.16
22	3	self	2.67	2.34	1.22	5.71	0.18	155.87	3.33	6.08
23	4	self	3.54	2.33	2.51	6.45	0.17	270.20	3.71	3.75
24	'Legend'		3.81	2.78	1.38	7.51	0.19	368.44	3.98	2.59
25	Exp. Hybrid		3.55	2.63	1.40	7.54	0.19	316.93	4.65	2.07
LSD			0.35	0.19	0.46	0.65	0.022	70.31	0.87	0.83
Mean			3.54	2.38	2.44	7.00	0.18	302.38	4.12	4.30

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

Table 24. Mean performance of entries at La Mesa, NM.

Entry	Parent 1	Parent 2	Bulb Trait[z]							
			BD	BH	C/B	R/B	RT	BW	SSC	PA
1	1	1	2.57	1.55	1.20	6.70	0.20	98.46	7.37	9.10
2	2	1	4.36	2.51	3.55	7.51	0.18	467.59	5.03	1.75
3	3	1	3.65	2.40	1.88	7.71	0.22	302.85	4.56	5.02
4	4	1	3.60	2.18	2.31	7.54	0.20	261.78	4.80	6.69
5	5	1	3.74	2.28	2.33	8.18	0.20	305.20	6.53	4.77
6	1	2	3.58	2.17	2.54	8.29	0.17	267.41	4.80	2.64
7	2	2	2.79	2.17	2.02	6.27	0.16	165.59	5.61	3.13
8	3	2	3.04	2.49	1.51	6.84	0.20	226.02	5.94	4.80
9	4	2	3.11	2.25	1.88	6.67	0.15	214.93	4.86	2.80
10	5	2	3.09	2.33	2.13	6.70	0.15	212.55	5.49	1.24
11	1	3	3.66	2.38	2.39	7.78	0.24	324.94	6.18	4.57
12	2	3	3.04	2.29	1.86	6.68	0.16	214.23	4.62	3.74
13	3	3	2.87	2.39	1.34	6.72	0.18	189.69	4.97	5.89
14	4	3	2.78	1.90	2.00	6.44	0.17	151.21	5.49	3.86
15	5	3	3.11	2.30	1.93	7.45	0.18	236.49	5.96	3.70
16	1	4	3.26	2.04	1.70	6.90	0.21	248.41	7.32	5.21
17	2	4	2.86	2.10	2.44	6.55	0.14	168.96	6.11	2.09
18	3	4	2.97	2.38	1.83	6.47	0.19	204.61	5.23	5.22
19	5	4	2.96	2.15	1.68	6.32	0.17	189.15	5.79	3.26
20	1	self	2.37	1.54	0.87	7.98	0.16	84.04	7.25	7.87
21	2	self	2.76	2.08	1.88	6.44	0.14	154.79	5.54	3.11
22	3	self	2.31	2.11	1.02	5.57	0.19	113.10	6.14	5.42
23	4	self	3.23	2.26	2.00	6.36	0.20	234.17	5.83	3.65
24	'Legend'		3.30	2.48	1.02	7.37	0.21	255.51	4.68	4.06
25	Exp. Hybrid 1		3.41	2.59	1.40	7.67	0.20	288.68	4.53	1.48
LSD			0.36	0.20	0.47	0.72	0.030	62.69	1.13	1.23
Mean			3.14	2.21	1.87	7.00	0.18	223.21	5.63	4.20

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

The maternal effect was significant for C/B in Uvalde; and BD, BH, and C/B in the combined analysis. The nonmaternal effect was significant only for BH in Uvalde; and BH and BW in the combined analysis. The only significant environmental interaction effect in the combined analysis was the GCA by environment effect for BH.

To determine the ability of breeders to predict hybrid performance, the importance of GCA and SCA was measured for each trait (Table 28). The closer the

calculated values are to unity, the better the predictability based on GCA. Different results were obtained in the two locations with BD, BW, and to a lesser extent with BH and C/B. The combined analysis generally gave an estimate equal to the average of the two environmental estimates except for with SSC. Traits with high predictability based on GCA included RT, and PA.

Although there were differences for which combining ability effects were significant in the two locations and the combined analysis, the direction of significant effects was the same for all but one trait (Tables 29-36).

Table 25. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown at Uvalde, TX.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Reps	2	0.02	0.00	0.35	0.40	0.0002	1186.79	0.04	0.69
Genotype	15	0.57***	0.18***	0.76***	1.10**	0.0026**	20664.18**	1.40	9.02*
GCA	3	0.46**	0.24***	0.81**	2.30**	0.0076***	10831.15	1.26	27.36***
SCA	6	1.11***	0.22***	1.26***	1.24*	0.0018	39779.64***	1.57	7.29
REC	6	0.19	0.10**	0.44*	0.30	0.0009	10825.25	1.24	1.23
MAT	3	0.12	0.07	0.79**	0.43	0.0004	3111.69	1.00	1.58
NMAT	3	0.30	0.14**	0.13	0.19	0.0013	19926.03	1.32	1.05
Error	30	0.09	0.03	0.14	0.36	0.0009	5477.78	0.82	3.61
R^2		0.76	0.76	0.73	0.63	0.61	0.67	0.48	0.57
% CV		9.57	7.69	19.33	8.53	15.78	31.55	16.22	44.19

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 26. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown at La Mesa, NM.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Reps	2	0.00	0.02	0.12	0.14	0.0001	270.04	0.69	0.91
Genotype	15	0.52*	0.14*	0.63**	1.25***	0.0014***	25018.30*	1.25	5.72***
GCA	3	1.05**	0.36**	1.29**	2.71***	0.0057***	30676.46	1.09	21.64***
SCA	6	0.51	0.10	0.81**	1.61***	0.0003	29103.20*	1.20	2.28
REC	6	0.27	0.08	0.14	0.15	0.0002	18104.32	1.38	1.19
MAT	3	0.41	0.12	0.21	0.20	0.0001	23615.83	1.22	2.24
NMAT	3	0.13	0.05	0.07	0.11	0.0004	12592.82	1.53	0.14
Error	30	0.22	0.06	0.22	0.29	0.0003	11132.64	1.24	0.98
R^2		0.54	0.56	0.60	0.68	0.68	0.53	0.35	0.75
% CV		13.27	10.27	18.71	7.78	9.88	34.53	28.20	21.95

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 27. Mean squares from the combining ability analysis of variance of 8 bulb traits of onion measured on progeny of a four parent diallel grown at Uvalde, TX and La Mesa, NM.

		Mean Squares[z]							
Source	**df**	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Environment	1	2.49***	0.30*	5.86***	0.01	0.0008	103784.91***	62.76	1.39
Reps (Env)	4	0.01	0.01	0.24	0.27	0.0001	728.41	0.36	0.80
Genotype	15	0.96***	0.27***	1.28***	2.03***	0.0035***	40772.92***	1.45	12.55***
GCA	3	1.21***	0.47***	1.84***	4.79***	0.0130***	30459.29*	0.36	47.22***
SCA	3	1.52***	0.29***	1.89***	2.57***	0.0016*	66227.41***	1.77	5.82*
REC	6	0.40*	0.15***	0.50*	0.20	0.0006	26609.30*	1.46	1.75
MAT	6	0.45*	0.13*	0.85**	0.19	0.0002	22161.58	0.53	3.38
NMAT	3	0.38	0.18*	0.16	0.21	0.0009	33191.09*	2.41	0.18
G X E	15	0.14	0.05*	0.15	0.29	0.0005	5000.84	1.15	2.19
GCA X E	3	0.30	0.13	0.25	0.22	0.0003	11048.32	1.98	1.78
SCA X E	6	0.10	0.03	0.17	0.28	0.0005	2655.43	1.00	3.76
REC X E	6	0.07	0.03	0.08	0.26	0.0006	2320.28	1.15	0.66
MAT X E	3	0.09	0.05	0.15	0.44	0.0004	4565.94	1.69	0.45
NMAT X E	3	0.04	0.01	0.04	0.09	0.0008	-672.24	0.44	1.00
Error	60	0.16	0.04	0.18	0.33	0.0006	8353.13	1.04	2.25
R^2		0.67	0.67	0.72	0.66	0.64	0.62	0.64	0.63
% CV		11.80	9.17	19.09	8.16	13.21	33.79	21.44	34.05

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 28. The relative importance of combining ability (GCA and SCA) in determining hybrid performance for a given bulb trait.

	Bulb Trait[z]							
Location[x]	**BD**	**BH**	**C/B**	**R/B**	**RT**	**BW**	**SSC**	**PA**
Uvalde	0.45[x]	0.68	0.56	0.79	0.89	0.35	0.62	0.88
La Mesa	0.81	0.88	0.76	0.77	0.97	0.68	0.64	0.95
Combined	0.62	0.76	0.66	0.79	0.94	0.48	0.29	0.94

[z] BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
[y] TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
[x] Values calculated using GCA and SCA mean squares (g_i and s_i respectively) from the analyses of variance using the formula $2\ g_i /(2\ g_i + s_i)$.

Table 29. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb diameter in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.21***	-0.10										
	2	-0.03	0.06	1 x 1	-1.07***	-0.73***	-0.90***						
	3	-0.08	0.00	1 x 2	0.54***	0.29	0.41***	-0.40**	-0.49*	-0.45***	-0.24*	-0.18	-0.21*
	4	-0.10	0.05	1 x 3	0.41***	0.26	0.33***	0.12	0.02	0.07	0.22*	0.10	0.16
				1 x 4	1.19***	0.92	1.06***	-0.14	-0.04	-0.09	0.02	0.09	0.05
NM	1	0.25**	-0.13	2 x 2	-0.36*	-0.10	-0.23						
	2	-0.25**	0.18*	2 x 3	-0.05	-0.11	-0.08	-0.01	0.14	0.06	-0.08	-0.10	-0.09
	3	-0.06	-0.05	2 x 4	0.23	0.01	0.12	-0.15	0.10	-0.02	-0.16	-0.09	-0.12
	4	0.06	0.00	3 x 3	-0.20	-0.19	-0.19						
				3 x 4	0.04	0.23	0.13	0.10	-0.05	0.03	0.15	0.00	0.07
Comb	1	0.23***	-0.12*	4 x 4	-1.47***	-1.16*	-1.31***						
	2	-0.14*	0.12*										
	3	-0.07	-0.03										
	4	-0.02	0.02										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 30. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb height in onion.

Parents				Crosses									
Loc[z]	**Parent**	**GCA**	**MAT**	**Cross**	**SCA**			**REC**			**NMAT**		
TX	1	-0.11***	-0.05		TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
	2	0.05	-0.01	1 x 1	-0.45***	-0.33**	-0.39***						
	3	0.12***	0.08*	1 x 2	0.17**	0.10	0.14**	-0.16*	-0.22*	-0.19**	-0.12*	-0.08	-0.10*
	4	-0.05	-0.02	1 x 3	0.21***	0.13	0.17***	0.05	0.01	0.03	0.17**	0.10	0.13**
				1 x 4	0.52***	0.42**	0.47***	-0.07	-0.02	-0.05	-0.05	-0.02	-0.04
NM	1	-0.07	-0.06	2 x 2	-0.15	-0.03	-0.09						
	2	-0.11*	0.08	2 x 3	0.01	-0.04	-0.01	-0.11	0.04	-0.04	-0.03	-0.02	-0.02
	3	0.17***	0.03	2 x 4	0.11	0.01	0.06	-0.08	0.09	0.00	-0.09	-0.06	-0.07
	4	0.01	-0.06	3 x 3	-0.07	-0.04	-0.06						
				3 x 4	-0.08	0.00	-0.04	0.24**	0.16	0.20**	0.14**	0.08	0.11*
Comb	1	-0.09**	-0.05	4 x 4	-0.55**	-0.43	-0.49**						
	2	-0.03	0.04										
	3	0.14***	0.05										
	4	-0.02	-0.04										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 31. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for number of centers per bulb in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.04	-0.18*										
	2	0.21**	0.25**	1 x 1	-1.02***	-0.61**	-0.81***						
	3	-0.24**	-0.12	1 x 2	0.76***	0.55***	0.64***	-0.55**	-0.20	-0.35*	-0.12	-0.05	-0.08
	4	-0.01	0.05	1 x 3	0.30*	0.07	0.19	0.12	0.17	0.14	0.18	0.09	0.13
				1 x 4	0.97***	0.59	0.78**	-0.28	-0.08	-0.18	-0.05	-0.04	-0.05
NM	1	0.10	-0.03	2 x 2	-0.31	-0.46*	-0.38*						
	2	0.09	0.12	2 x 3	-0.33*	-0.16	-0.24*	0.25	0.10	0.17	-0.12	-0.13	-0.12
	3	-0.34***	-0.10	2 x 4	0.19	0.53	0.36	0.20	0.18	0.19	-0.01	0.08	0.04
	4	0.16	0.01	3 x 3	-0.12	-0.22	-0.17						
				3 x 4	0.28	0.52	0.40	-0.10	-0.15	-0.13	0.06	-0.03	0.01
Comb	1	0.07	-0.10	4 x 4	-1.44***	-1.64**	-1.53***						
	2	0.15*	0.18										
	3	-0.29***	-0.11										
	4	0.07	0.03										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 32. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for number of rings per bulb in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.40***	0.08										
	2	-0.06	-0.21	1 x 1	-1.11***	-1.18***	-1.15***						
	3	0.02	0.04	1 x 2	0.45*	0.79***	0.64***	0.37	0.05	0.18	0.09	0.06	0.06
	4	-0.36**	0.09	1 x 3	0.43*	0.17	0.30*	0.14	0.02	0.08	0.11	0.08	0.10
				1 x 4	1.32**	1.40***	1.37***	-0.21	0.01	-0.10	-0.20	-0.14	-0.16
NM	1	0.43***	0.02	2 x 2	-0.53	-0.16	-0.36*						
	2	-0.30**	0.03	2 x 3	0.04	-0.32	-0.15	-0.33	0.01	-0.16	-0.08	0.06	-0.02
	3	0.10	0.08	2 x 4	0.57	-0.15	0.22	-0.12	0.15	0.02	0.17	-0.01	0.08
	4	-0.23*	-0.13	3 x 3	-0.24	0.05	-0.09						
				3 x 4	0.00	0.05	0.02	-0.03	0.36	0.17	0.02	0.14	0.08
Comb	1	0.42***	0.04	4 x 4	-1.89**	-1.30*	-1.61***						
	2	-0.17*	-0.08										
	3	0.05	0.06										
	4	-0.30***	-0.02										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 33. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for ring thickness in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.019**	0.003										
	2	-0.023***	-0.005	1 x 1	-0.021	-0.013	-0.017*						
	3	0.008	0.005	1 x 2	-0.011	-0.002	-0.006	-0.008	-0.002	-0.005	-0.016	-0.002	-0.009
	4	-0.003	-0.003	1 x 3	0.025*	0.006	0.015**	0.015	0.000	0.008	0.017	0.000	0.009
				1 x 4	0.027	0.023	0.025*	0.005	-0.003	0.001	-0.001	0.001	0.000
NM	1	0.019***	-0.001	2 x 2	0.016	0.002	0.009						
	2	-0.016***	-0.001	2 x 3	0.007	0.005	0.006	-0.022	0.007	-0.008	-0.013	0.007	-0.003
	3	0.004	-0.001	2 x 4	-0.028	-0.006	-0.017	-0.005	-0.013	-0.009	-0.003	-0.009	-0.006
	4	-0.007*	0.003	3 x 3	-0.019	-0.006	-0.012						
				3 x 4	0.006	0.001	0.003	0.012	0.003	0.008	0.004	0.008	0.006
Comb	1	0.019***	0.001	4 x 4	-0.004	-0.018	-0.011						
	2	-0.020***	-0.003										
	3	0.006	0.002										
	4	-0.005	0.000										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 34. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for bulb weight in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	29.1*	-13.9										
	2	-1.6	15.6	1 x 1	-197.9***	-175.9***	-186.8***						
	3	-4.5	-1.8	1 x 2	99.7***	65.3	84.3***	-96.7**	-120.5**	-110.3***	-67.2**	-46.2	-57.6**
	4	-23.1	0.1	1 x 3	82.0**	58.2	69.2**	43.9	26.0	35.0	55.9*	46.7	50.3**
				1 x 4	214.0***	228.3**	220.2***	-2.7	-27.5	-15.1	11.3	-0.5	7.3
NM	1	39.3*	-30.5	2 x 2	-69.0*	-31.4	-50.1						
	2	-47.2*	43.7*	2 x 3	-9.0	-10.7	-10.8	-9.0	35.9	13.5	-26.4	-17.7	-24.0
	3	7.9	-9.8	2 x 4	47.2	8.2	26.7	-25.4	18.6	-3.4	-40.8	-28.6	-33.7
	4	0.1	-3.5	3 x 3	-40. 8	-53.5	-49.1						
				3 x 4	8.5	59.3	39.7	27.6	22.8	19.4	29.6	29.1	26.4
Comb	1	33.7**	-22.6	4 x 4	-269.7***	-295.9**	-286.6***						
	2	-24.9*	30.1*										
	3	2.2	-7.2										
	4	-11.0	-0.2										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 35. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for soluble solids content in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	0.23	0.29										
	2	-0.25	0.00	1 x 1	1.14	0.44	0.78						
	3	-0.14	-0.10	1 x 2	-0.88	0.06	-0.42	-0.13	-1.15	-0.64	-0.41	-0.61	-0.51
	4	0.16	-0.19	1 x 3	-0.19	-0.81	-0.49	0.47	-0.07	0.20	0.08	0.29	0.21
				1 x 4	-1.20	-0.14	-0.65	0.82	0.05	0.43	0.34	0.32	0.30
NM	1	-0.29	-0.29	2 x 2	0.60	0.00	0.29						
	2	0.20	0.25	2 x 3	0.23	0.18	0.22	-0.41	-0.21	-0.31	-0.51	-0.40	-0.43
	3	-0.02	0.07	2 x 4	-0.55	-0.23	-0.37	0.30	0.07	0.18	0.10	-0.21	-0.08
	4	0.10	-0.02	3 x 3	-0.20	0.27	0.07						
				3 x 4	0.37	0.09	0.13	-0.34	-0.02	-0.08	-0.43	-0.11	-0.22
Comb	1	-0.02	0.00	4 x 4	1.39	0.28	0.89						
	2	-0.01	0.13										
	3	-0.09	0.01										
	4	0.12	-0.13										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Table 36. Estimates of general combining ability (GCA), maternal (MAT), specific combining ability (SCA), reciprocal (REC), and nonmaternal (NMAT) effects for pyruvic acid content in onion.

Parents				Crosses									
Loc[z]	Parent	GCA	MAT	Cross	SCA			REC			NMAT		
					TX	NM	Comb	TX	NM	Comb	TX	NM	Comb
TX	1	1.33***	-0.06										
	2	-1.26***	-0.12	1 x 1	2.35**	-0.06	1.09*						
	3	0.14	0.39	1 x 2	-1.00	0.69*	-0.08	0.36	0.41	0.26	0.30	-0.03	0.07
	4	-0.21	-0.21	1 x 3	-1.48*	-0.90**	-1.20***	-0.23	-0.40	-0.32	0.22	-0.14	0.07
				1 x 4	-2.21	0.34	-0.90	-0.36	0.43	0.03	-0.52	0.17	-0.14
NM	1	1.29***	0.11	2 x 2	1.07	0.07	0.52						
	2	-0.73***	-0.33	2 x 3	0.31	-0.27	0.01	-0.38	-0.69	-0.53	0.14	0.01	0.05
	3	0.14	0.37*	2 x 4	-1.45	-0.56	-0.97	0.24	-0.23	0.01	0.16	-0.05	0.02
	4	-0.70***	-0.15	3 x 3	0.35	1.09*	0.74						
				3 x 4	0.46	-1.01	-0.29	0.97	0.39	0.69	0.36	-0.13	0.12
Comb	1	1.33***	-0.01	4 x 4	3.20	1.23	2.16*						
	2	-0.98***	-0.20										
	3	0.12	0.39*										
	4	-0.47*	-0.18										

[z]Locations: TX = Uvalde, TX; NM = La Mesa, NM; Comb = Combined location.
*, **, *** Significant at P < 0.05, 0.01, and 0.001 respectively.

Heterosis

Estimates of better parent heterosis (BPH) were measured for all hybrids in the diallel cross (Tables 37, 38). These estimates ranged from -53% to 107% in Uvalde and from -44% to 196% in La Mesa. Trait averages for heterosis ranged from -13% to 45% in Uvalde and from -16% to 62% in La Mesa. The greatest heterosis was seen for C/B and the greatest negative heterosis was seen for SSC.

When the hybrids are grouped according to type of parent, interesting trends appear (Table 39). The hybrids from DH parents show the greatest heterosis for all traits except C/B, RT, and SSC (and PA in Uvalde). The hybrids from conventional inbred parents generally gave the lowest heterosis. The hybrids between DH and conventional inbred parents, with DH mothers, performed better in Uvalde than their reciprocal crosses. The reverse was true in La Mesa.

Table 37. Heterosis estimates of crosses grown at Uvalde, TX.

Entry	Parent 1	Parent 2	Better Parent Heterosis[z] BD[y]	BH	C/B	R/B	RT	BW	SSC	PA
2	2	1	25.03	12.19	58.39	15.10	-13.55	107.08	5.25	74.29
3	3	1	20.14	-0.46	43.70	7.51	0.48	47.99	-21.23	-17.28
4	4	1	13.67	2.29	30.97	8.40	-0.64	4.45	0.93	30.44
6	1	2	16.21	8.92	67.64	15.33	-12.54	70.07	-52.99	67.43
8	3	2	-6.47	-9.67	30.37	-10.33	-7.71	-15.71	-0.27	29.65
9	4	2	-5.03	-3.93	12.15	-7.55	-4.33	-10.88	-11.13	2.15
11	1	3	21.11	0.20	62.34	7.34	1.50	67.09	-37.02	-30.16
12	2	3	1.42	-7.75	47.34	-11.66	-0.51	10.19	-13.28	3.73
14	4	3	2.53	-11.59	85.43	-6.76	-3.63	6.84	-9.11	-16.54
16	1	4	13.49	1.52	28.07	12.30	-3.54	20.48	9.99	45.76
17	2	4	-4.08	-1.31	21.81	-2.44	-16.13	-5.83	-9.22	-14.10
18	3	4	0.28	2.00	57.44	0.03	-1.42	26.58	-15.13	17.36
		Mean	8.19	-0.63	45.47	2.27	-5.17	27.36	-12.77	16.06

[z]Calculated as BPH = ((F_1 – BP)/100) X 100.
[y]BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

Table 38. Heterosis estimates of crosses grown at La Mesa, NM.

Entry	Parent 1	Parent 2	Better Parent Heterosis[z] BD[y]	BH	C/B	R/B	RT	BW	SSC	PA
2	2	1	56.05	15.55	196.04	12.02	-10.35	182.38	-31.68	-44.20
3	3	1	27.23	0.67	56.64	14.83	9.40	59.65	-38.14	-14.86
4	4	1	11.63	-3.44	92.88	12.44	1.71	11.79	-34.85	83.12
6	1	2	27.99	-0.23	111.67	23.73	-17.42	61.49	-34.83	-15.73
8	3	2	5.94	4.33	12.78	1.92	11.48	19.15	5.91	53.42
9	4	2	-3.72	-0.36	-6.02	4.91	-22.02	-8.22	-16.65	-10.65
11	1	3	27.53	-0.33	99.52	15.90	17.05	71.30	-16.10	-22.47
12	2	3	5.91	-3.91	38.82	-0.46	-12.78	12.93	-17.59	19.52
14	4	3	-13.72	-20.37	49.23	-4.03	-15.88	-35.43	-5.95	5.64
16	1	4	1.04	-9.52	42.08	2.92	7.68	6.08	-0.59	42.53
17	2	4	-11.42	-6.90	21.75	3.14	-26.96	-27.85	4.74	-33.21
18	3	4	-8.07	-0.14	36.07	-3.71	-3.67	-12.62	-10.40	42.75
		Mean	10.53	-2.05	62.62	6.97	-5.15	28.39	-16.34	8.82

[z]Calculated as BPH = ((F_1 – BP)/100) X 100.
[y]BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).

Table 39. Ranking according to better parent heterosis estimates.

Location	Hybrid Type[y]	Rank[z] BD	BH	C/B	R/B	RT	BW	SSC	PA
Uvalde	DH X DH	1	1	3	1	4	1	4	4
	CI X CI	4	4	4	4	1	3	2	1
	DH X CI	2	2	2	2	3	2	3	2
	CI X DH	3	3	1	3	2	4	1	3
La Mesa	DH X DH	1	1	4	1	4	1	4	1
	CI X CI	4	4	2	4	3	4	2	3
	DH X CI	3	3	3	3	2	3	1	2
	CI X DH	2	2	1	2	1	2	3	4

[z]BD = bulb diameter (in.), BH = bulb height (in.), C/B = centers per bulb, R/B = number of rings per bulb, RT = ring thickness (in.), BW = bulb weight (g), SSC = soluble solids content (brix), and PA = pyruvic acid content (µM pyruvate/ml).
[y]Parents of hybrids were either doubled haploids (DH) or conventional inbreds (CI).

DISCUSSION

Crosses

The reduction of the diallel to four parents limits the amount of useful information that can be gleaned from this experiment. Eleven parents would have been comparable to what is seen in the literature (Christie and Shattuck, 1992). Although it would also have been analyzed as a completely fixed model, the resulting combining ability estimates would have been based on the entire DH group to date at Texas A&M University and their parent lines. The current experiment is only representative of a sub-sample of the group. As a result, the combining abilities obtained can only be used as a preliminary guide to determine appropriate breeding and selection strategies for this material.

The conventional inbred line I11, parent 4 in the diallel, and its A-line pair I11A were removed from the field design to include the two commercial checks. At the time, it was believed that the self (entry 23) would give an accurate representation of line I11. Although this appears to be true for the DH parents and their corresponding selfs, it is not the case for the other conventional parent (Tables 23, 24). As mentioned previously, when comparing entries 13 and 22, they differ significantly for five of the eight traits measured. The values measured for the self were always lower than those of the parent line. However, when comparing entries 22 and 23 it appears that perhaps the latter is not so prone to inbreeding depression after one generation of selfing. Although a direct test to determine how appropriate the substitution of entry 23 for line I11, the values from this entry were utilized in the diallel analyses for line I11.

Variation

There were several factors which contributed to increased variation in the experiment. The first factor was a last-minute change in location which resulted in the transplants going to La Mesa spending an extra month in the flats. It is unknown what effect this extra time in the flats would have on the bulb traits measured. The location change also resulted in differences in how the plots were set up (80" beds vs. every other bed on 40" beds) and irrigated (drip vs. furrow). Any variance between locations, caused by the difference of time in transplant trays and the differences in plot set-up, would be separated out in the ANOVA and become part of the environmental component.

The second factor was damage to transplants that occurred during transport to Uvalde. The flats, containing all of the transplants for both locations, collapsed on top of each other causing significant damage to the onion plants. Although there was a difference in the extent of the damage on each flat, it was not possible to quantify this difference. The damage resulted in uneven stands in Uvalde, due to the death of some plants, and a reduction in the number of plants per plot in both locations. This factor would have increased within plot variation and could also have contributed to some variation between certain plots.

The third factor was a problem with weeds and harvester ants in Uvalde. The weeds were a result of midseason rains which prevented access to the plots for weed removal. During this time, harvester ants came in from an adjacent uncultivated field and cut off leaves and sometimes entire plants from areas near their tunnels. The

number of weeds growing tended to increase in a direction parallel with the blocks. That means that its effects would likely be included in block variance. It would only increase variance between plots if the entries responded differently to the increased competition. The randomness of the ant damage would probably result in its effects being contributed to experimental error.

The fourth factor was the weather during the growing season in La Mesa. The weather was cooler than average for most of the growing season, with a sudden spike in temperature towards the end of the season. This dramatic shift in temperature occurred almost one month prior to harvest, resulting in the rapid maturity of the onions. Because the later maturing crosses were not very far into the bulb expansion phase of their growth cycle, they were likely affected more than early maturing crosses. This likely contributed to variation in yield between the two locations, which would be separated out in the ANOVA and become part of the environmental component.

The fifth factor was the nature of the crosses. Because no male sterility was available in these DH lines, all crosses performed were fertile by fertile crosses. As a result, the seed produced by the female plant in each cross contained some seed that was the result of self pollination. In the crosses in which the two parents were different colors, the selfed individuals are easy to distinguish and discard. However, in crosses involving parents of the same color, it was not possible to separate out these individuals. The amount of self pollination in the different colored crosses was calculated for each cross. For all crosses included in the field experiment the range of self pollination was from 24% to 96%, with an average of 67% overall. For the crosses included in the

diallel the range of self pollination was 27% to 85%, with an average of 66% overall. The inclusion of selfed progeny with the hybrids would tend to increase the within variance in these plots and would make the entry appear more like the female parent than it really is, resulting in an inflated maternal effect.

Mean Performance

Two comparisons among the parent lines are of interest. First, comparing the performance of the selfed lines to their parent pair; second, comparing the performance of the DH line (H11) to its conventional line equivalent (I11). Since I11 was not included in the field study, H11 can be compared to the self (23). The selfs were statistically similar to their parent pair except with R/B and RT in both locations for the first pair (1, 20); C/B and RT in Uvalde for the second pair (7, 21); and BD, BH, R/B, BW, and SSC in both locations for the third pair (13, 22). In Uvalde, H11 gave lower values than the self of its conventional line equivalent for all traits except SSC. However, none of the differences were statistically significant except for BD. In La Mesa, H11 gave lower values for all traits except C/B. Differences between the two lines were significant for BD, RT, and BW.

Two comparisons can also be made with the hybrids. The first is the comparison of the average performance of the DH by DH hybrids and the DH by conventional inbred hybrids. In Uvalde, the hybrids between DH parents had greater averages for all traits except BH and SSC. In La Mesa, the same trends were true except that the RT was equal between the two groups. The second comparison is the average hybrid performance versus the average parent performance. In Uvalde, the average hybrid

performance was greater than the average parent performance for all traits except SSC and PA. In La Mesa, average hybrid performance exceeded average parent performance except for RT (the two groups were equal), SSC, and PA.

When comparing mean performance values, it must be kept in mind that lower values for C/B and PA are desirable for commercial onions. Unfortunately, all of the hybrids had significantly greater C/B than the commercial checks. Although the number of centers per bulb exceeded commercially acceptable levels in many of the crosses, it must be realized that all but one of the parent lines (I2, 7) also displayed this trait. Although the appearance of some hybrids with lower PA than their parents is promising, none of the values were statistically different than their parents.

Combining Ability

The presence of significant environmental variance components for four of the eight traits, and a GCA by environment effect for one trait, shows that duplicate trials in separate locations or years would be necessary with these lines. This is valuable information for a breeding program because it allows the breeder to determine the best way to allocate available time and money.

Apparent trends existed between the GCA effects of a line in the different bulb traits, but only for the DH parents. If the line tended to increase BD in its progeny, it also increased C/B, R/B, RT, BW, and PA. This relationship is not surprising, but shows the inappropriateness of using these lines as parents to create hybrids: the goal of onion breeding is to reduce C/B while increasing traits related to bulb size. It is possible that the appearance of a trend in the DH parents, but not in the conventional inbred parents is

due to the very small number of parents used in the study. Further studies comparing the differences between DH lines and conventional lines could be very valuable.

The ability to predict the performance of a hybrid by the general combining ability of its parents would be very valuable to a plant breeder. This ability would allow the breeder to determine which hybrid crosses to make without extensive testing. Also, the breeder could cross the best combiners to create improved breeding populations from which to select new parents. For the lines in this study, prediction of hybrid RT and PA should be quite reliable. For BD and BH, the value was high for La Mesa but not for Uvalde. The lack of predictability is due to the effects of environment on the crosses included in this study for the traits measured.

Heterosis

The trends in the heterosis estimates of these hybrids are in agreement with the theory of heterosis. The levels of heterosis would be expected to be greatest in the DH by DH hybrids, followed by DH by conventional inbred hybrids, conventional inbred by DH hybrids, and then conventional inbred by conventional inbred hybrids. This is because part of the heterosis observed when inbred lines are crossed is due to the recovery of lost vigor due to inbreeding depression

It is interesting to note that the one deviation, from the expected levels of heterosis in the different types of hybrids, was the performance in La Mesa of the hybrids between a DH and a conventional inbred line. Those with DH mothers showed less heterosis than those with conventional inbred mothers. The exact reason for this reversal of the expected trend in one location, but not in the other, is not known. It may

be that the shortened season and different day length in La Mesa affected the two types of parent lines differently. It may also simply be a result of the limited number of parents and hybrids included in the study.

Implications for Breeding

To be a useful parent in a breeding program, a line must have good mean performance as well as good combining ability. Some of the lines used in this study matched this requirement for some of the traits. For example, H1 (parent 1) was comparable to the commercial checks for BD and also had significant positive GCA and SCA effects in Uvalde. In breeding, however, being good for one trait is not sufficient. A line must have good performance and good combining ability for multiple traits.

The lines and hybrids in this study had the most problems with C/B. This trait is very important in the industry. One of the lines in this study showed promise with this trait. Line I2, 7 (parent 3) was comparable in its C/B to the commercial checks and also gave significant negative GCA estimates, which is good for this trait. It also gave negative SCA estimates when combined with line H11 (parent 2). This finding is very important because it shows that DH lines can be useful parents in a breeding program. Even if DH by DH hybrids cannot be found that are commercially acceptable, DH by conventional inbred hybrids are also potential candidates. The benefits of using a DH line as one of the parents is an increased uniformity in the hybrids, a trait which is very important to the industry.

CHAPTER VI

CONCLUSION

Seven doubled haploid (DH) lines developed at Texas A&M University were evaluated using five published sequence-tagged microsatellite (STMS) markers. These lines were also evaluated using twenty four amplified fragment length polymorphism primer (AFLP) combinations. Four of the five STMS markers showed a high amount of variability between the DH lines. They were also able to detect remnant heterozygosity within the conventional inbred sister lines and the original parent lines. However, the markers showed no variation within the DH lines, confirming the phenotypic and cytogenetic evidence of their homozygosity. The AFLP markers, on the other hand, were not very polymorphic in this material. Although some useful polymorphisms were found, especially the one differentiating between H6 and H8, they were not able to generate enough information to compare a DH line to its corresponding conventional inbred and their original parent lines.

A complete diallel crossing scheme involving the seven DH lines and four conventional inbred sister lines was attempted. Not all of the crosses could be made, however, and so the experiment was divided into two four-parent diallels for which all crosses had been completed. The first diallel included only DH parents; the second diallel included two DH lines and two conventional inbred lines. One of the conventional inbreds in the second diallel was a sister line to one of the DH lines used in the study. Eight bulb traits were measured on a sample of individual plants in two

environments with three repetitions per line. Individual plant data was used to determine mean performance for each bulb trait. Plot means were used to obtain combining ability estimates and heterosis estimates (second diallel only).

The mean performance and combining ability analyses revealed that the DH lines evaluated would not be useful as parents in a breeding program. The primary problem with most of the hybrids with a DH parent was the number of centers per bulb. This is a common problem in onion breeding in general, and was a problem with most of the parent lines of the DH lines used in this study. Although it is likely that this problem could be overcome, it would require stringent selection of parental material for this trait and/or the development of a large number of DH lines from which to select. The former would require many years for the development of uniform single center parent lines. The latter would require a sizeable investment of time and money to develop a large number of DH lines, especially given the low regeneration frequency. It is unlikely that the creation of DH lines would be more efficient than traditional breeding methods in the development of improved cultivars. However, it may prove useful in long-term inbred development by reducing the sensitivity of germplasm to inbreeding depression. Further testing would be needed to determine if improved lines can indeed be selected after random crossing among DH lines. Also, such a program would need to ensure that sufficient care is taken to preserve genetic variability by carrying forward DH material from a large portion of the breeding material.

Many factors in the field experiments contributed to the amount of variability in the experiments. However, despite the large amount of variation, significant differences

between entries did exist for almost all of the traits measured. These data can be used to determine how these lines could be used in a breeding program. In addition, most of the crosses have been made for the larger diallel including all of the DH lines and their conventional inbred sister lines. With the completion of a few more crosses the hybrids from the larger diallel could be used to obtain information on all of these lines.

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APPENDIX

A1. TABLE OF 24 AFLP PRIMERS USED IN MARKER SCREEN

Primer Combination	Selective Primers[z]	
	E+3	M+4
1	CAA	CACA
2	CAA	CAGC
3	CAA	CACT
4	CAA	CACG
5	TGA	CACA
6	TGA	CAGC
7	TGA	CACT
8	TGA	CACG
9	TAC	CACA
10	TAC	CAGC
11	TAC	CACT
12	TAC	CACG
13	CTG	CCCA
14	CTG	CCCT
15	CTG	CCCC
16	CTG	CCCG
17	ACT	CCCA
18	ACT	CCCT
19	ACT	CCCC
20	ACT	CCCG
21	GTC	CCCA
22	GTC	CCCT
23	GTC	CCCC
24	GTC	CCCG

[z]Contain appropriate adapter sequences and indicated selective bases.

A2. NUMBER OF BULBS MEASURED FROM EACH CROSS IN DH FIELD TRIAL

	Uvalde			La Mesa		
Entry	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	10	10	10	10	10	10
2	2	3	0	10	2	10
3	8	10	10	10	10	10
4	6	9	5	1	7	3
5	10	10	6	10	10	10
6[z]	10	10	10	10	10	10
7	7	10	10	10	4	6
8	10	9	10	10	10	10
9	10	10	10	10	10	10
10	2	6	1	3	2	1
11	7	8	10	10	10	10
12	10	10	10	10	10	7
13	3	7	6	10	10	9
14	10	10	10	10	10	10
15	2	10	5	4	7	10
16	9	6	5	10	10	10
17	10	10	10	10	10	10
18	10	10	10	10	10	10
19	3	4	5	2	3	1
20	10	9	10	10	10	10
21	10	10	10	10	10	10
22	2	1	1	4	6	4
23	6	5	8	10	10	10
24	10	10	10	10	10	10
25	7	4	3	10	10	10
26	10	10	10	10	10	10
27	10	10	10	10	10	10
28	10	10	10	10	10	10
29	0	1	1	3	1	2
30	1	2	0	4	3	10
31	10	7	10	10	9	10
32	10	10	10	10	10	10
33	10	10	10	10	10	10
34	10	10	10	10	10	10
35	8	6	10	10	10	10

[z]Bold entries included in diallel

A3. NUMBER OF BULBS MEASURED FROM EACH CROSS IN DH BY CONVENTIONAL INBRED FIELD TRIAL

	Uvalde			La Mesa		
Entry	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1[z]	8	10	8	10	10	10
2	1	1	0	4	7	2
3	8	10	10	10	10	10
4	10	8	6	8	6	6
5	9	9	10	10	10	10
6	5	5	9	10	4	1
7	5	4	4	10	10	4
8	5	1	5	10	10	7
9	10	8	8	10	4	3
10	10	10	10	10	6	4
11	1	2	3	9	7	7
12	10	10	10	8	7	7
13	10	10	10	2	10	10
14	9	8	8	3	10	10
15	2	1	1	10	9	10
16	7	5	8	10	10	9
17	10	9	10	6	10	10
18	10	10	10	10	10	10
19	10	10	10	9	8	10
20	10	7	10	10	10	10
21	10	8	7	10	5	10
22	9	10	6	10	6	10
23	10	10	9	10	10	10
24	10	10	10	10	10	10
25	10	10	10	10	10	10

[z]Bold entries included in diallel

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